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The Importance of Lipoids in Nutrition

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THE IMPORTANCE OF LIPOIDS IN NUTRITION

BY

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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ENTITLED THE IMPORTANCE OF LIPOIDS IN NUTRITION

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

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IMPORTANCE OF LIPOIDS IN NUTRITION.

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IMPORTANCE OF LIPOIDS IN NUTRITION

I. INTRODUCTION.

1. PURPOSE OF EXPERIMENTS.

The real necessity of having lipoids in food has caused a great deal of discussion and argument among workers in nutrition. The question is still more or less unsettled and for this reason these feeding experiments were conducted in an effort to see if lipoids were really necessary. There is very good evidence for and against the idea of the necessity of lipoids in food, both for maintainance and for growth. If the experiments showed that the lipoids were necessary, the next purpose of this work was to determine which fraction of the lipoids was indispensable.

The question, as to whether lipoids are really necessary in foods, is of great dietetic importance. In feeding children, which are dependent for food upon some other source than their mothers milk, prepared foods, it is very essential that the lipoid content of the diet should be large enough. Many babies, receiving substituted milks, are barely living or maintaining themselves upon their foods. Now if babies like these were given foods which contain large amounts of lipoids in addition to their maintainance food, it is highly possible that they would begin growing. The fact that no prepared babies food causes so rapid or large a growth as the mothers milk seems to be a well known fact even though the patent milk Co's. sometimes advertise differently. Now it may be that this lack on the part of the prepared milk is due in part at least to the deficiency of lipoid or lipoid-like material. Of course, this work does not take up this question with babies, still some very important conclusions can be drawn from the mice

experiments. Not only is this experiment, with lipoids in foods important in prepared babies food, but it is likewise important in food given to grown people. Older persons must have lipoid material in their foods as well as the young ones, for this substance or substances found in the lipoid fraction of foods is necessary for maintainamce as well as growth.

II HISTORICAL DISCUSSION:

The various constituents of a synthetic food have each been worked with in an endeaver to find out just which and how much of a food are required in a perfect diet. Most of the older experiments were done with natural foods while the present more scientific idea is to use artificial synthetic foods made from purified materials.

A. INORGANIC:

The inorganic constituents of a food are very important. It must not only have the proper qualitative constituents, but the quantities used must also be in the proper proportions. The question of salts is one that must be settled in order to experiment with any of the other constituents.

Phosphorus is an essential part of the salts. It has been shown by McCollum[12] that collapse follows the feeding of extremely low or phosphorus free foods. During starvation the bones were drawn from. The inorganic phosphorus supplied the need just as well as the organic phosphorus contained in Phytin. Taylor,[13] Forster[14] and Lunin[15] have all shown that phosphorus is absolutely necessary.

Lunin[15] showed that sulphates were obtained from the oxidations of the sulphur in proteins when all the inorganic sulphur was absent so that sulphur in the salts does not seem to be necessary, although it is always found in natural foods in the organic as well as in the inorganic form.

The study of the iron used in nourishment has been extensively studied by Soccin[16]. He draws the following conclusions from his experiments. (1) The inorganic iron compounds are split

the intestine by bacteria. (2) Mice die without iron. (3) Injected iron is thrown off immediately in the urine and feces. (4) Inorganic iron compounds of egg-yolk are reabsorbed. These experiments show conclusively that inorganic iron is needed in salt mixtures.

In an attempt to make up a salt mixture which was adequate for synthetic food Röhmann[32] used the following mixture.

Calcium Phosphate	10 grams.
Potassium hydrogen phosphate	37 "
Sodium chloride	20 "
Sodium citrate	15 "
Magnesium citrate	8 "
Calcium lactate	8 "
Iron citrate	2 "

He thought this was adequate, but it has later been shown by the work of Wheeler[5] that the mixture was not sufficient for animal food.

Osborne and Mendel[1] made up a salt mixture as near like their successful Protein-free-milk (to be discussed later) as possible and found it to be successful. It consisted of the following:

Hydrochloric acid	12.75 grams.
Phosphoric acid	10.32 "
Citric acid	10.10 "
Sulphuric acid	.92 "
Calcium carbonate	13.84 "
Magnesium carbonate	2.42 "
Potassium carbonate	14.13 "
Sodium carbonate	14.04 "
Iron citrate	.634 "

The inorganic salts that compose the successful salt mixture of McCollum[32] are:

Sodium chloride	.146 grams.
Magnesium sulphate	.225 "
Sodium di hydrogen phosphate	.293 "
Potassium mono " "	.805 "
Calcium tetra " "	.456 "
Iron lactate (Merck)	.100 "

2.4 grams of this and 1.3 grams calcium lactate with each 100 grams of ration.

Most successful of all the salts yet used is Osborne and Mendel's[4] Protein free milk. The objection to this salt is the fact that it is not a synthetic mixture. In order to purify such a natural salt it is therefore necessary that it be extracted. It was prepared by these observers by adding hydrochloric acid to centrifugated milk. The precipitated casein was filtered on a cheese-cloth and the nearly clear solution was filtered through a pulp filter. The filtrate was then purified further by the alternate treatment of alkali and acid. The filtered milk was then boiled a few minutes and then filtered. After being neutralized with sodium hydroxide, it was evaporated on the steam bath at 70°.

B. PROTEIN:

The proteins have probably received more thought than any other constituent in food. Gelatine[10] as a single protein in food has been shown to be inadequate. Likewise, Zein[9] lacks tyrosine and tryptophane and for this reason it is not an adequate single protein. Similarly gliadin fails to permit growth in white rats.

Casein on the other hand is an adequate protein [1, 2,

3, 4, 5, 6, which can be used as the sole protein in food. Casein does not contain all of the amino acids required in the body, for it lacks glycocoll, This acid can be synthesized, (1) in the body and therefore the casein does not need it. Various amounts of casein have been tried for the best production of growth. 18% casein in the food gave best results[1].

C. CARBOHYDRATES:

The carbohydrates which are used in these feeding experiments are starch and lactose. Both of these substances can be obtained in a pure state and are fully adequate for the carbohydrate part of foods. 1, 4, 5.

D. LIPOID FATS AND EXTRACTIVES:

The parts of food which have recently caused the greatest amount of discussion are, fats, lipoids, and extractives. This question, however, is an old one, and dates back to 1881 when Lunin[15] thought that there must be some unknown substance in milk. Of course he had no idea what the substance was, still, he could not explain his results without the use of this unknown substance. Ten years later, Socin[16] got a similar idea; when his artificial foods failed to keep the animal in proper nourishment. He explained his failure by the fact that an artificial food must take in some unknown mixture of compounds in order for the animal to live.

At the present time it is in regard to the necessity of this fraction, called, lipoids, that Osborne and Mendel disagree with Stepp. Stepp[18] used a food made of rice meal soaked in milk for feeding his mice. He designated this food as milch-protamol. Mice live and grow easily upon this mixture. In order to get rid of the lipoids in this food, it was necessary to extract with alcohol and

ether. He found that after such treatment the mice soon died. Butter added to this extracted food, was not able to keep them alive. By the addition of various fats to the extracted Milch Protamol he shows definitely that the fats were not able to prevent death. He also added Lecithin and cholesterin and these substance could not maintain the mice. Then he added the original extract of the Milch protamol and then his mice lived.

In the above mentioned work Stepp noticed that food plus heated milk did not serve the animals as well as did the addition of cold milk. This led him to study the effect of heating foods [20]. He came to the following conclusions. (1) Alcohol and ether extracts of various foods loose nutritive powers by two days heating. (2) Some of the lipoids present in food are labile. (3) Two days heating with water is also disasterous. (4) Addition of cold extracted lipoids saves the animals. Hence some lipoids are changed by boiling. (5) Duration of boiling is an important factor. (6) Mice cannot build lipoids from the simple "Bausteine".

Further work along the same line [19] has brought Stepp to the conclusion that alcohol extracts all the lipoids necessary for life. In this work be added a mixture of lipoids, consisting of lecithin, cholestein, kephalin, cerebron and phytin, which were not able to compensate for the substances removed by the alcohol and ether extract.

Osborne and Mendel fed their rats upon food which was lipoid free by extraction with ether [24]. Hot alcohol was not used in the extraction as it was assumed that the ether removed all fats or fat-like substances. With this ether extraction their experiments gave positive evidence of the despensability of true

fats for growth. Concerning the addition of butterfat to protein free milk (untreated) [25] Osborne and Mendel conclude that " It thus appears improbable that glycerides of the fatty acids ordinarily present in foods are responsible for the promotion of the growth observed when butterfat replaces lard in the diet of rats, which have ceased to grow. Lecithin and other phosphorous and nitrogen containing substances are excluded by the absence of phosphorous and nitrogen from our butterfat and cholesterol by the fact that lard contains more of these than does butter." The weakness in these experiments seems to be in the fact that a natural protein free milk was used which was not extracted.

McCollum and Davis [33] while working with rats got a growth, after they had ceased to grow upon artificial foods, by adding the ether extracts of egg yolk or butterfat.

The question as to whether skimmed milk contains lipoids is a disputed question Vladimir Nzegovo [17] says that they are not present while Schlossmann says it is found in milk. The question is undecided at present but the whole difficulty is probably an error in the method of determining the lipoid contents.

McCollum [27] fed hens a mixture of skimmed milk and rice meal, the latter was extracted twice with boiling alcohol. Ground limestone and sand was also supplied the chickens. This food was practically free from lecithin and fat. These hens increased in weight 33-36%. He concludes that it is evident that the phosphatids are readily synthesized when the hens are fed rations free from these substances.

In the experiments cited above the results would have been much more reliable if the milk powder had been extracted be-

fore use.

Hens were later used by McCollum [28] in experiments and here he used 30% Merrill Soules skimmed milk powder with 70% polished rice, extracted with boiling alcohol. Two extractions were made using a liberal amount of 95% alcohol in boiling for 20 - 30 minutes. The yolks of the eggs laid by the hens in this experiment contained 30% of lecithin and 6.39% of kephalin. This fact also leads him to believe that there must be a synthesis of these two phosphatids.

Funk [29] obtained a substance from rice polishings which cures beri-beri and polyneuritis in birds. This substance comes out in the alcoholic extract and is termed by him as Vitamine. The substance absent from polished rice but present in the polishings is an organic base precipitated by phosphotungstic acid. This substance may be related in some unknown way to the substances which are designated as lipoids by some authors.

Suzuki [22] obtained a hot alcohol extractive which he calls Oryzannins. The substance is found necessary for chickens, pigeons, mice and dogs fed upon foods extracted with alcohol. Every food which lacked this substance extracted from the rice husks were unable to live. Animals eating artificial food have to have the Oryzannins to live.

All the work done so far seems to point to the fact that there is a substance in the food which is absolutely necessary to life is not known yet and it may be some lipoid or phosphatid substance or it may be some unknown extractive like Funk's Vitamine.

2. IDEAL FOOD FOR EXPERIMENTAL PURPOSES.

The ideal food for experimental purposes would be a chemically pure synthetic food. Carl Voit [11] says " Es wäre unstreitig am besten, konnte man(zur Untersuchung der Grundtatsachen des Stoffwechsels) nur reine chemishe Verbindungen(die reinen Nahrungsstoffe), Z. B. reines Eiweiss,, Fett, Zucker, Starkemehl, Aschenbestandteile oder gemische derselbem, geben. Da aber die Menschen und auch die Tiere nur selten solche geschmacklose Gemenge auf die Dauer aufzunehmen oder zu ertragen vermögen, so bleibt für die meisten Fälle nichts anderes übrig, als schon durch die natur, zusammemgesetzte Mischungen(Nahrungsmittal) zu wählen. Doch wäre es wohl möglich und ganz verdienstvoll, die grundversuche, nachdem vorher der Weg mit letzteren Mischungen gefunden worden ist, mit den reinen Nahrungsstoffen zu wiederholen, obwohl sich dabei sicherlich im wesentlichen Keine anderen Resultate ergeben werden."

Osborne and Mendel have this same idea in view because all of their foods are made up of pure materials as far as it is possible to do so.

A. VIEWS HELD BY VARIOUS OBSERVERS AS TO PALALABILITY.

One of the main troubles in the past with these synthetic foods[31] has been their lack of palatability. However later work points toward the correctness of the statement made by Wheeler[5] that the monotony of diet appears to be of little importance, as a detriment to the nutrition of white mice, provided the diet is an otherwise sufficient one. Anorexia seemed always to follow rather than to precede a loss of weight and to be the result rather than the cause of the decline.

Osborne and Mendel[30] state that failure to eat by animals

on experimental foods is probably due to the monotony of the food and perhaps to lack of bacteria in the intestines.

McCollum[12] seems to be pretty certain that the failure of artificial foods in feeding experiments lies in the fact that the food lacks palatability. He says that growth is produced if the foods are changed often. And along this same line he finds that young animals are much easier to adapt themselves to rations possessing a low degree of palatability than old animals. It seems at the present time that artificial foods can be used successfully in feeding experiments although these mixtures are not perfect as compared to natural foods. The foods do not seem to be as appetizing as natural substances, yet the animals as shown in different authors' work already mentioned, are able to eat enough food to grow to a size almost if not as great as the animals with natural food products.

III EXPERIMENTAL.

1. ANIMALS USED.

The animals used in these experiments were albino mice. They are especially fitted for this kind of work, because they are easy to handle. They seldom bite and, after being handled a few times in the process of weighing they become extremely gentle. They do not seem to mind being picked up by the tail when it becomes necessary to weigh them. White mice are large enough to begin an experiment after two weeks at which time they weigh 10 to 12 grams. They are sexually mature at two months and fully grown at 150 days or less(according to Miss A.E.C. Lathrop of Granby Mass.) Mice are omnivorous and for this reason they can eat most any kind of food given them. Another point to be considered in the choice of animals is to get sensitive animals and a white mouse is very quickly influenced by changes in food.

Mice are comparatively cheap, selling for fifteen dollars per hundred. They are quick breeders and plenty of mice can be kept on hand if the proper precautions are taken.

Another important factor in feeding experiments with synthetic food is the amount of food used by the animals. Mice eat very little in a day, seldom 10 grams. This saves not only the expense of the experiment but also the work in preparation of food.

The mice were kept in cages made of fine mesh wire with an open top. The mice were supplied with filter-paper bedding, and crystallizing dishes to hold their foods. Fresh clean water was furnished, at all times by means of bottle placed in a slanting position on top of the cage. A glass tube with the end drawn

out allowed a drop of water to hang in their proximity continually. The mice were weighed upon the pan of a large balance. They were kept upon this pan by covering it with a large glass crystallizing dish.

The mice which were not being fed experimental food and which were merely used for breeding purposes were fed a mixture of ground corn and sunflower seeds. Dog biscuit was kept in the cages at all times and, once a weak, carrots and meat were given them.

2. PREPARATION OF INGREDIENTS OF FOODS.

The ingredients used in the artificial foods were prepared as pure as possible with the main object in view of keeping everything lipoid-free except where it was added intentionally.

A. INORGANIC SALTS.

In the preparation of the inorganic constituents of the food Röhmanns, Osborne and Mendell's and McCollum's[35] salt mixtures were prepared as indicated in their original preparation of the mixtures. Protein free milk was obtained from skimmed milk. It was first diluted about a fourth with water. Then it was precipitated with constant stirring with the least amount of dilute hydrochloric acid. The white flocculent precipitate was then allowed to settle and the supernatent liquid was filtered off. This filtrate was warmed up and another precipitate of protein filtered off. The solution was then neutralized with sodium hydroxide. The liquid was then slowly evaporated on the water-bath. The protein free milk was then made lipoid-free by extraction with Benzine 60%, absolute alcohol 40%, for three days. Each day the benzine and alcohol were filtered off the milk. Then in order to

get rid of any traces of Benzine which might be present the salts were washed with ether three times followed by drying in an air-dryer.

The Merril -Soule skimmed milk powder consists of the following:

Butterfat	1.81
Casein	32.31
Albumin	5.85
Milk sugar	49.32
Ash	8.21
Moisture	2.

This product is nothing more than skimmed milk with a very low moisture content. Since it was shown in preliminary experiments that Benzine remained in milk even after it was washed several times with alcohol and ether with repeated drying in the air-dryer, a new method of extraction had to be adopted. This consisted in boiling the powder with ether for two days. Very little fat like material was removed. Then the powder was boiled with alcohol for three days. Here a great deal more was extracted. Between each treatment the residue was dried and passed through a forty mesh sieve.

B. CASEIN.

The casein used was a product of Sargent and Co. and was not in a powdered form but in small particles. Ether dissolved a yellowish greasy substance out of it which was precipitated by acetone. This casein was treated on a water bath for three periods of, forty eight hours each with 95% alcohol. Then the residue was boiled twice with ether for one day. The Casein was filt-

ered, pressed, dried and then sieved before each treatment. The last extracts when evaporated showed no sign of phosphatid or fats being present.

C. LARD.

The fat used was lard of the best variety. It was completely soluable in ether. A qualitative test was run for Nitrogen according to the usual method. This test was negative. A Kjeldahl was also run and no Nitrogen was found. Besides the lard mentioned above Olive oil and butter were also used. A good grade of olive oil and creamery butter was employed.

D. CORBOHYDRATES.

The carbohydrates fed in the experiments consisted of Starch and Lactose. A phosphorous test was run upon each of these and they both proved negative. Hence there is no chance for a phosphated being present in the carbohydrates.

C. LIPOIDS OF DRIED EGG YOLK.

The lipoids which were obtained from egg yolk, were extracted from the material dried upon glass plates in the air dryer without heat. The egg yolks were treated with hot ether for one day after which time the solution was filtered off. The ether extract was then treated with twice its volumn of absolute alcohol and a precipitate came down. This is egg Kephalin. The filtrate was then evaporated and taken up in ether. The egg lecithin was then precipitated with Acetone. The filtrate was evaporated and called the third fraction.

Egg yolk was also extracted with hot alcohol. The hot filtrate was filtered into a dish surrounded by ice and a preciptate come down. This was then filtered. The first preciptate will

be called insoluable in the cold alcohol fraction and the latter will be called the soluable in the cold. Other portions of egg yolk were extracted with both alcohol and ether and is called, on this account, the alcohol and ether extract.

A part of the egg yolk was extracted with cold alcohol and cold ether separately. The egg-yolk after treatment with cold alcohol was warmed in the presence of alcohol and here another fraction was precipitated.

F. ALCOHOLIC SOLUABLE LIPOIDS OF BRAIN TISSUE.

In order to try other lipoids than egg-yolk, some brain tissue was extracted with cold alcohol and the filtrate evaporated.

3. RESULTS.

The first five preliminary experiments run were with benzine and alcohol, extracted casein and milk powder. These mice became sick and died within ten days time. The toxic effect of the benzine was later shown by foods in which benzine was not used. Without the benzine extract, the mice having the same foods lived in some cases over 100 days.

Mouse No. 6.

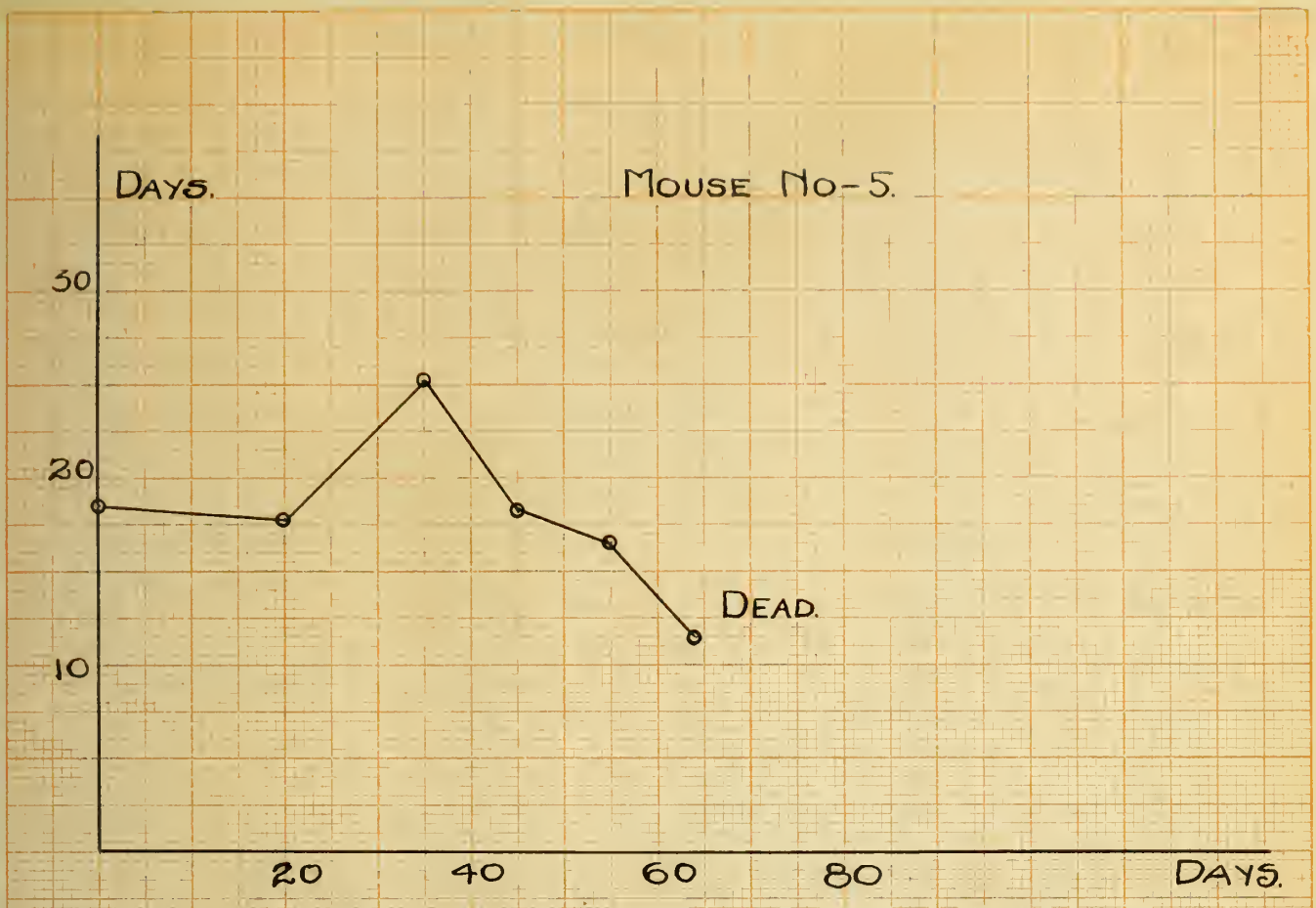
Food. Material	gms.	Weights. days	gms.
Alc. & Ether ext. casein(pp 15)	18	1	10.5
Untreated Milk Powder(pp 14)	22	20	16.55
Treated Milk Powder(pp 14*)	22	35	19.2
Corn Starch	23.8	45*	20.5
Lactose	9.2	55	19.5
Röhmann Salts(pp 4)	1.	62	17.6
Lard	25.	69	15.
Dried Egg Yolk	1.	76	12.9
		80	10.

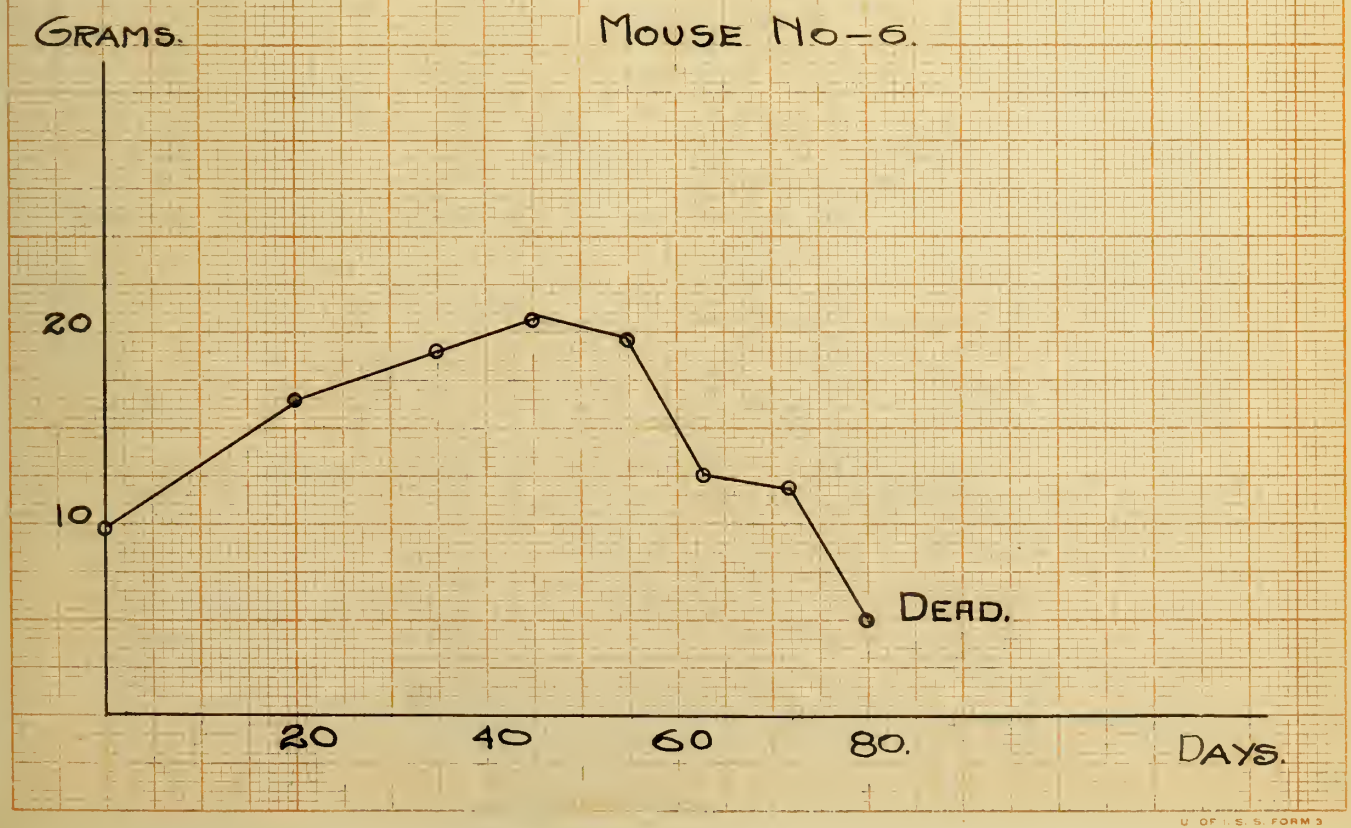
*represents the day upon which the extracted milk powder was fed. The amount of lipoid in one gram of egg-yolk is extremely small. When one remembers that the necessary fraction is only a small part of the whole lipoid extract, then it is not hard to understand why the gram of egg-yolk in the whole food mixture was not sufficient for even maintainance.

Mouse No. 5.

Food.		Weights.	
Material.	gms.	days	gms.
Alc. & Ether ext. of Casein	18	1	18.5
Untreated Milk Powder	22	20	17.35
Treated Milk Powder	22	35	25.5
Corn Starch	23.8	*45	18.5
Lactose	9.2	55	16.5
Röhmann's Salts	1.	62	11.6
Lard	25.		
Lecithin(pp 15)	.5		

Mouse No. 5, with the lecithin extract of egg-yolk, failed entirely to maintain its weight after the milk powder was extracted. Lecithin is not the vital substance.





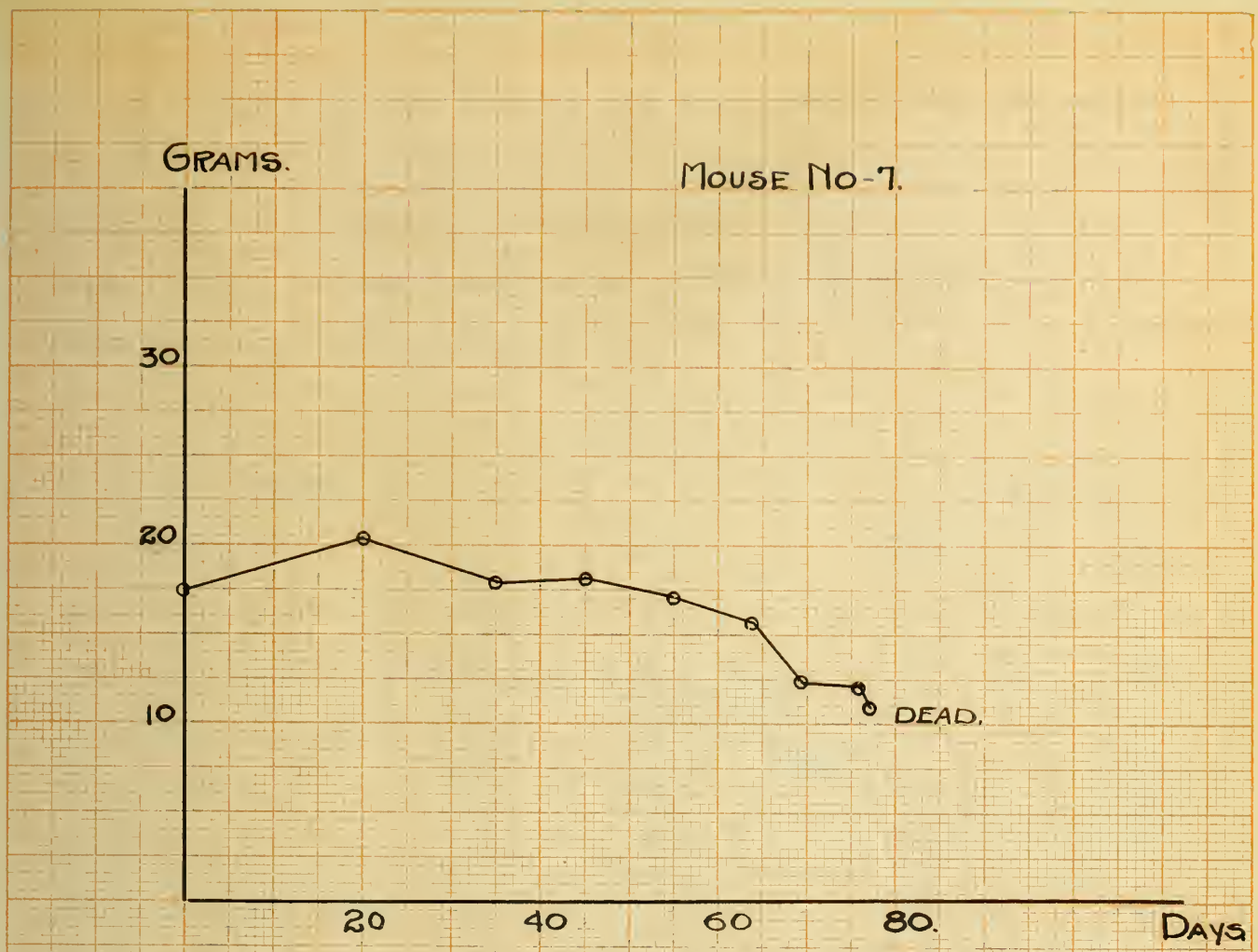
Mouse No. 7.

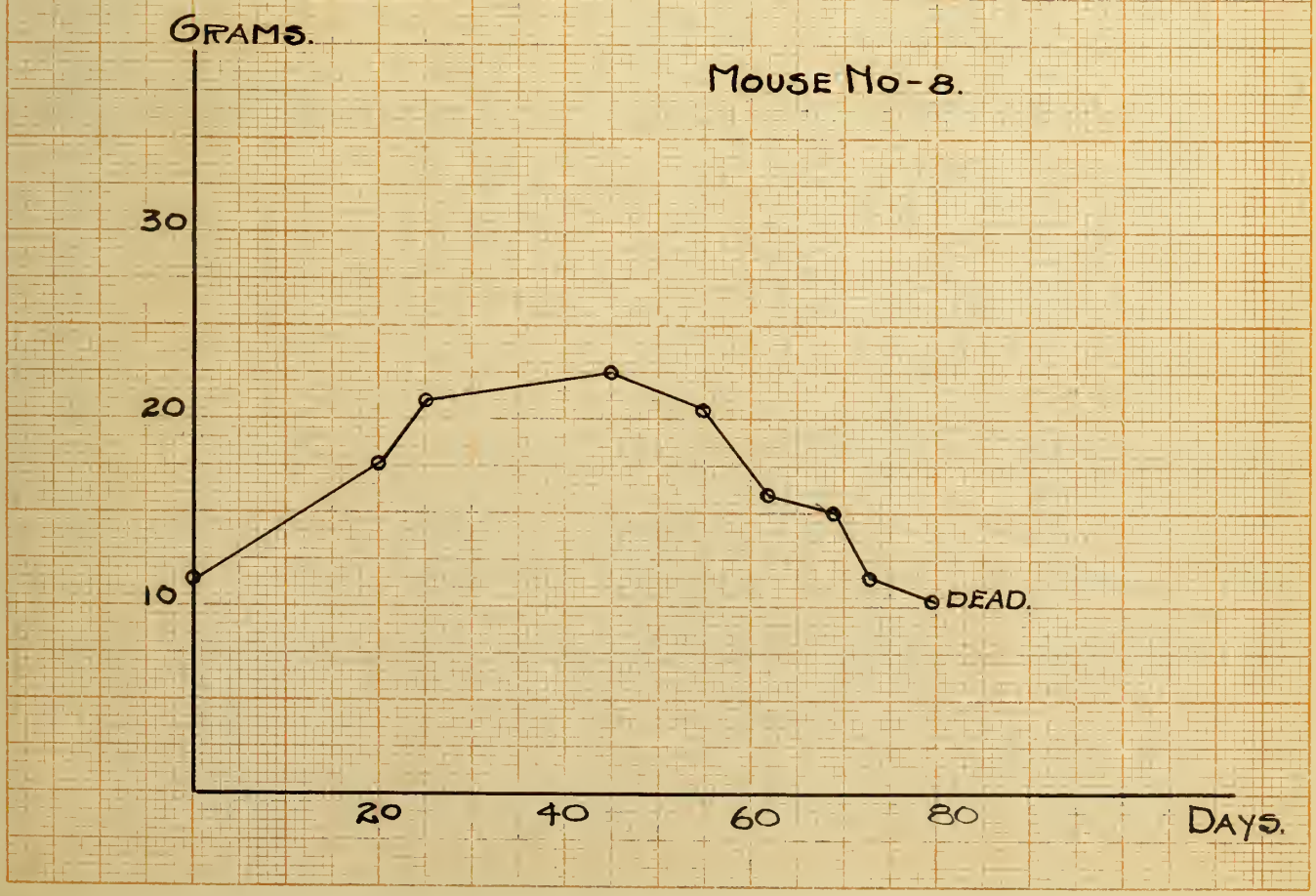
Food.		Weight.	
Material	gms.	days.	gms.
Ether & Alc. ext. Casein	18	1	17.5
Milk Powder	22	20	17.85
Corn Starch	23.8	35	18.
Lactose	9.2	45*	18.1
Röhmann's Salts	1.	55	17.
Lard	25	62	15.8
Kephalin(pp 15)	.5	69	12.5
		76	12
		77	11

Kephalin did not supply the necessary substance taken out in the extraction of the milk powder. The mouse lived longer than did No. 5 although not quite as long as No. 6.

Mouse No. 8.

Food		Weight.	
Material	gms.	days	gms.
Ether & Alc. ext. Casein	18	1.	11.5
Milk Powder	22.	20.	17.85
Corn Starch	23.8	35	21.5
Lactose	9.2	45*	22.5
Röhmann's Salts	1.	55	20.5
Lard	25	62	15.9
3rd fraction(pp 15)	.5	69	15.
		73	11.5
			dead



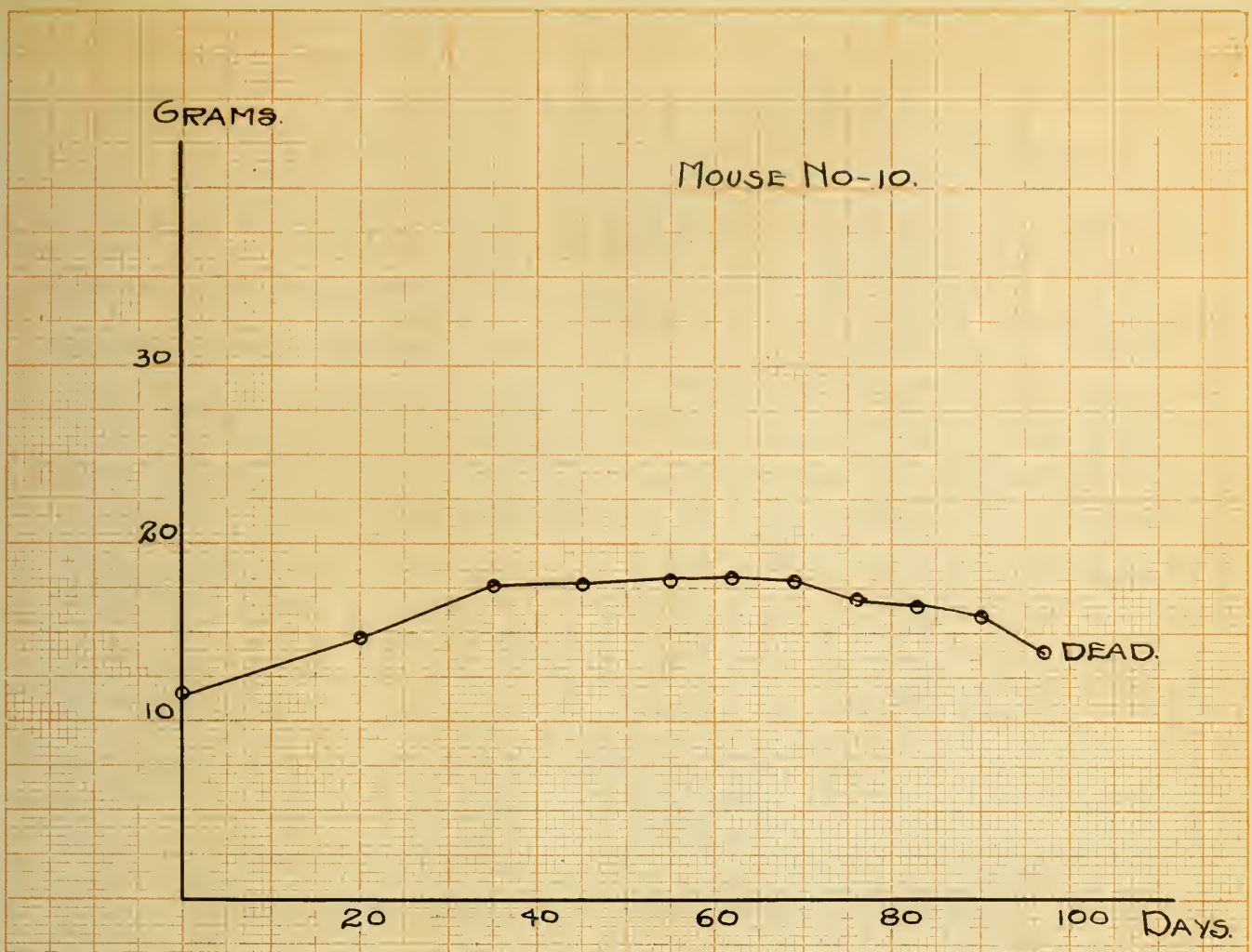


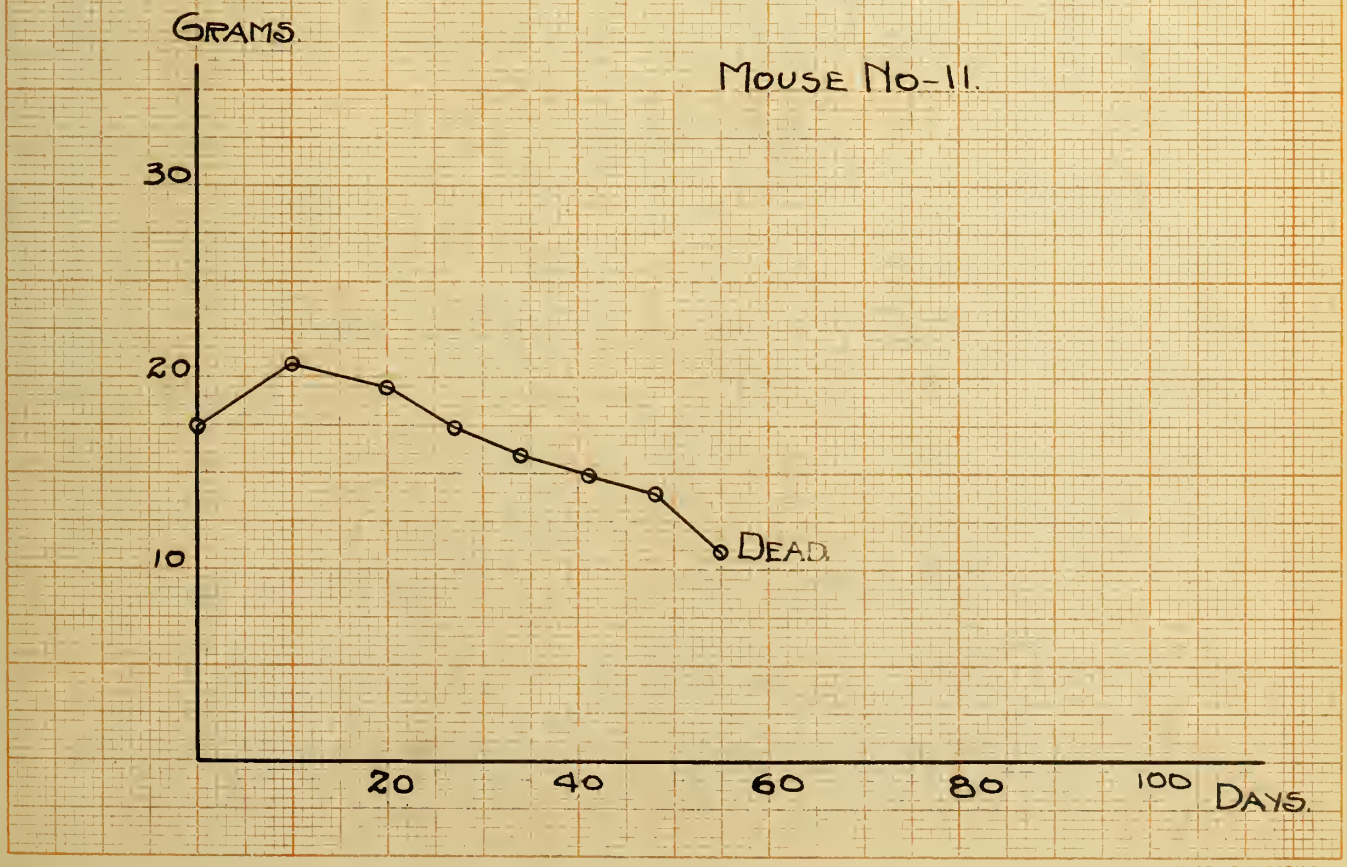
The food was evidently not made sufficient for growth or even maintainance by the addition of the 3rd fraction. This mouse like the others already described became emaciated. Their hair became rough and greasy-looking and just before death they became so weak that they could not move.

Mouse No. 10.

Food.		Weights.	
Material	gms.	days	gms.
Alc. & Ether ext. Casein(pp 15)	18	1	11.5
Untreated Milk Powder(pp 14)	22	20	14.8
Treated Milk Powder(pp 14)	22	35	17.7
Corn Starch	23.8	45*	17.8
Lactose	9.2	55	18.
Röhmann's Salts(pp 4.)	1.	62	18.2
Lard	25	69	18.
Ether & Alc. ext. of Egg-Yolk(pp 15)	.5	76	17.
		83	17.
		90	16.4
		97	14.
		102	dead.

This mouse excells all the others in the series in growth and maintainance easily. It is noticed that it did not stop growing when the milk powder was treated with alcohol and ether. This seems to show that the alcohol and ether extracts contain the substance removed in the extraction of the milk powder.





Mouse No. 11 (control)

Food.		Weight.	
Material	gms.	days	gms.
Alc. & Ether ext. Casein	18.	1	17.8
Untreated Milk Powder	22.	10*	20.17
Treated Milk Powder	22.	20	19.5
Corn Starch	23.8	27	17.5
Lactose	9.2	34	16.2
Röhmann's Salts	1.	41	15.4
Lard	25.	48	14.5
No extractives.		55	11.dead

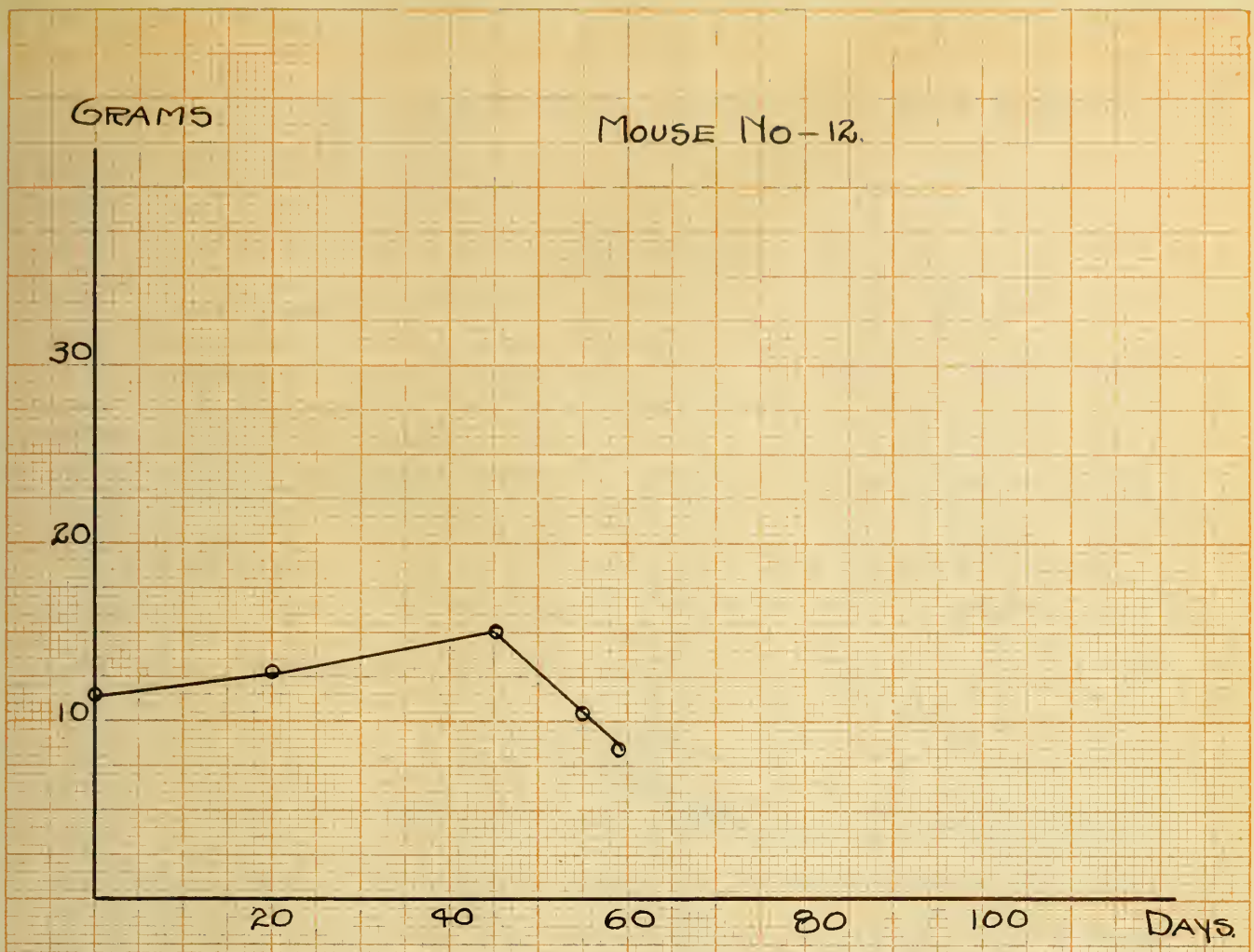
*This milk powder was treated on the tenth day.

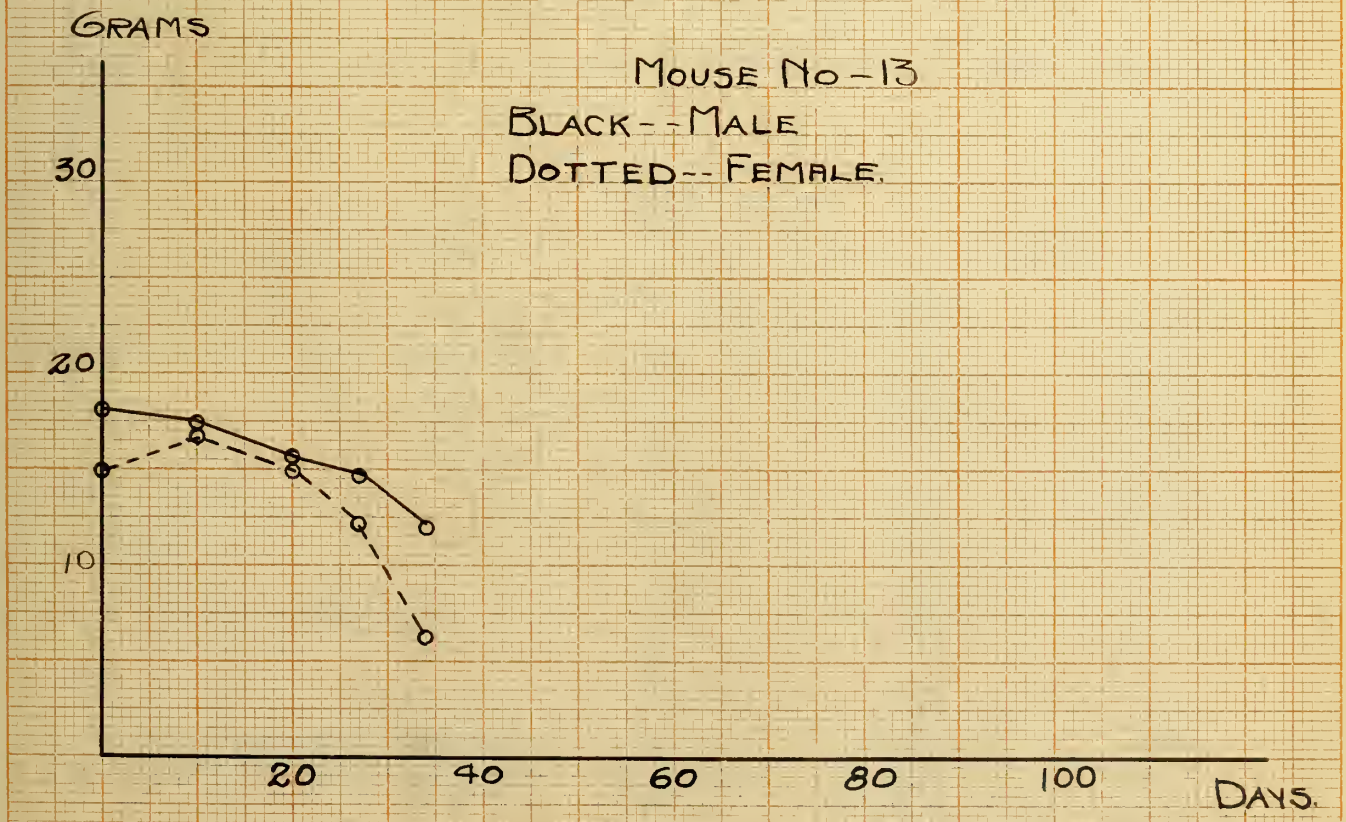
This control mouse did not grow nearly so well as did the others, although it did not get untreated milk powder for so long a time. Some necessary extractive has been removed which is absolutely necessary for the welfare of the animal.

Mouse No. 12.

Food.		Weight.	
Material	gms.	days	gms.
Ether & Alc. ext. Casein	25	1	11.5
Corn Starch	23.8	20	15.4
Lard	25.	45	15.
Lactose	24.	55	10.5
Salt Mixture Röhmann	1.	59	8.7 dead

No milk powder or phosphatids were given this mouse in its food and its lack of growth and final death are well shown. The quick death in comparison to previous experiments points toward the





fact that not only is some extract absent but that the salt mixture is not the proper kind.

Mouse No. 13.

Food. Materials	gms.	Weight. days	male	female
Ether & Alc. ext. Casein	18	1	18.2	15.
Ether & Alc. ext Milk P.	22	10	17.5	16.9
Corn Starch	38.8	20	15.8	15.
Lactose	19.2	27	14.85	12.2
Salt Mixt. Röhmann's.	1.	34	12	6.2
			dead	dead

This is a fat and lipoid free diet. The weights show a continual loss until death. There are two possible causes for this quick decline either the fat is necessary, at least better present, or the phosphatids are important constituents of the food that are missing. This mouse, at death, was the most dejected looking animal imaginable.

Mouse No. 14.

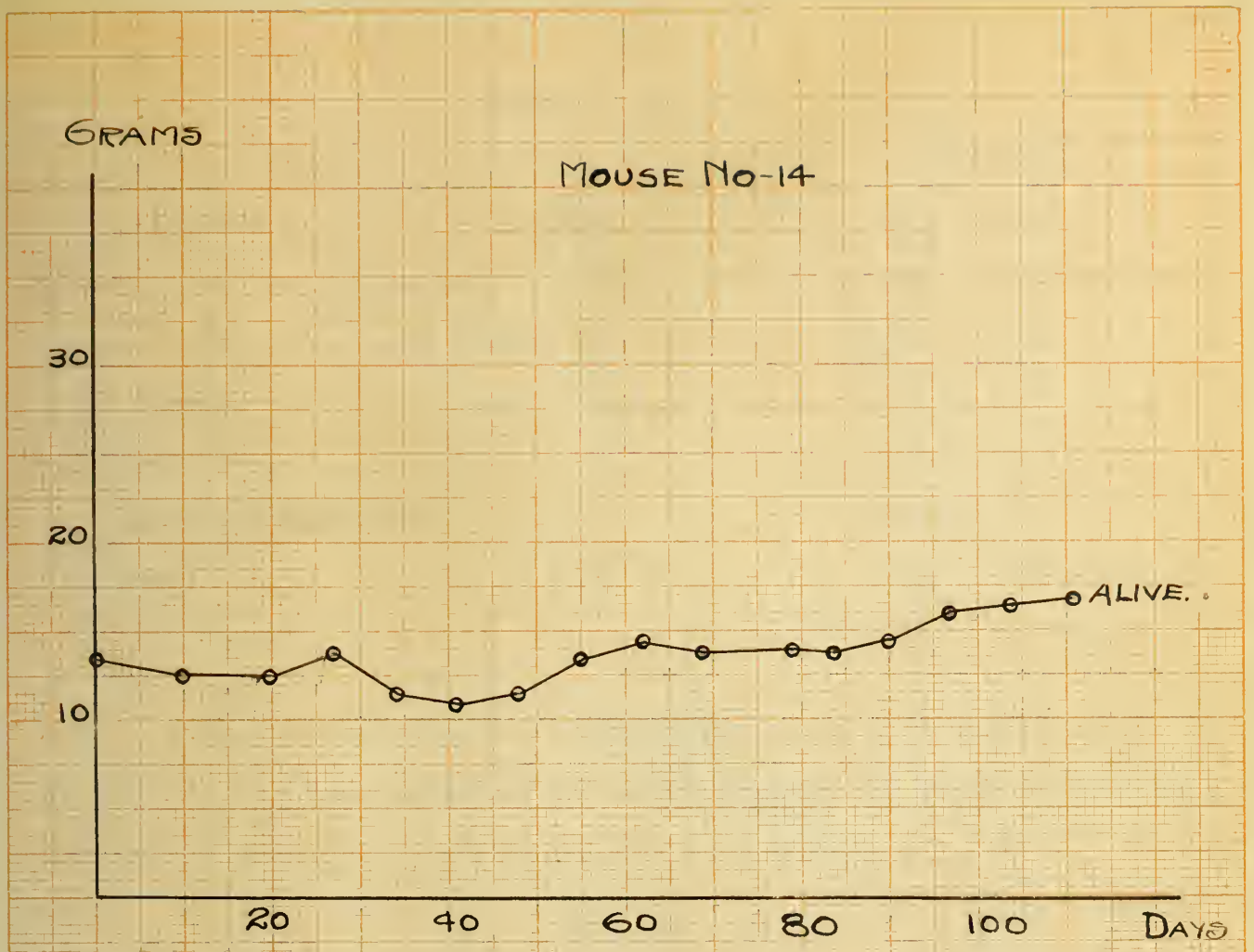
Food. Material	gms.	Weight days	gms.
		1	12.7
Ether & Alc. ext. Casein	18	10	12.5
		20	12.5
Ether & Alc. ext. Milk Powder	22	27	12.9
		34	11.5
Corn Starch	23.8	41*	11.3
		48	12.1
Lactose	9.2	55	13.3
		62	14.3
Salt Mixture Röhmann's	1.	69	13.7
		79	14.
Lard	25	83	14.
		90	14.8
Alc. & Ether ext. Egg-yolk		97	15.6
*added on 41 day (pp 15.)	3	104	15.7
		111	15.9
		Still alive, looking fine.	

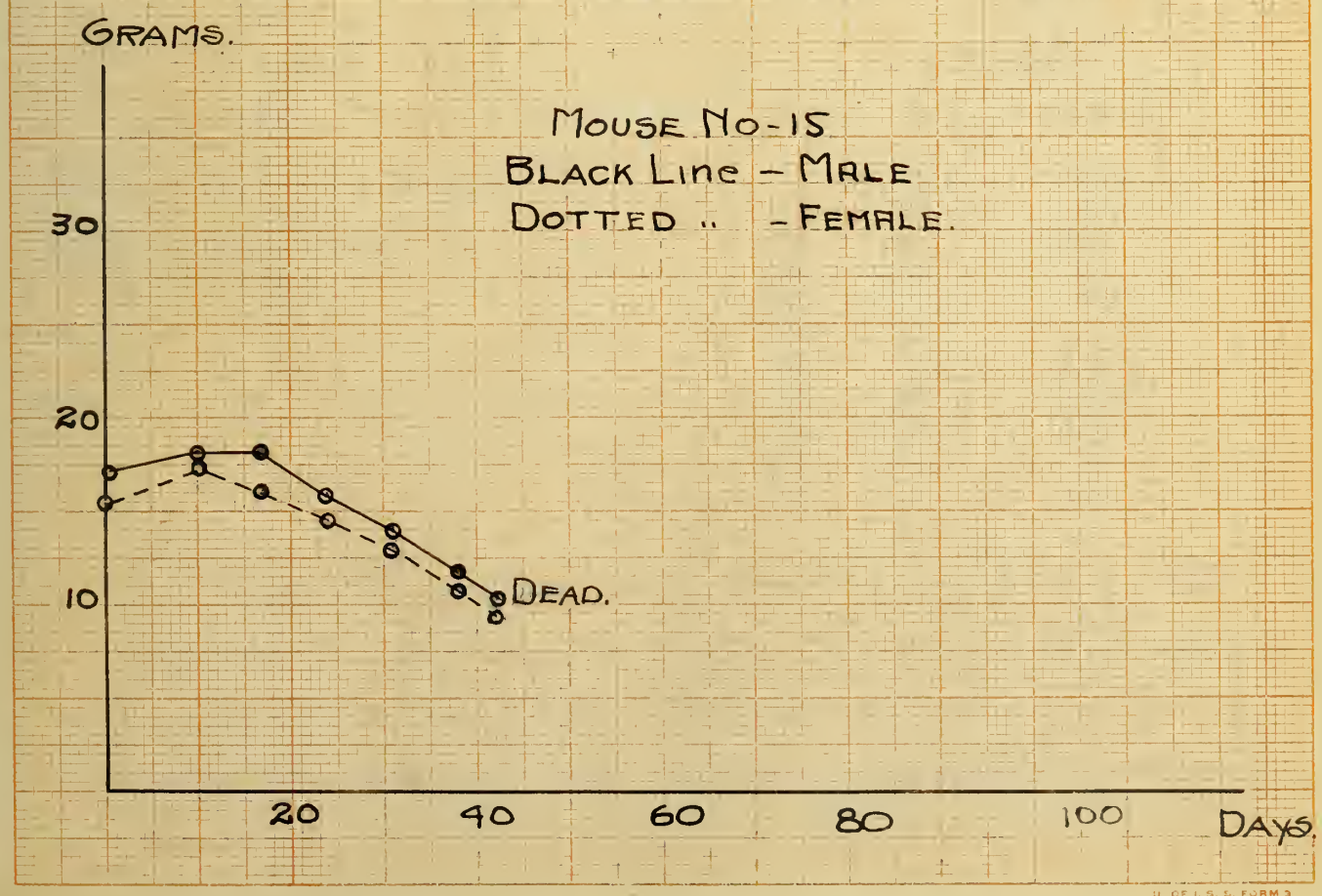
This mouse is receiving the same food as No. 13 except it is getting a larger quantity of alcohol and ether extract of egg-yolk. This is the most successful mouse that was fed upon ether and alcohol extracted milk powder. The important thing to note in this experiment is the fact that the mouse was declining in weight until the phosphatids were added and then a gradual increase takes place. This mouse is still in a perfect condition and is just like the control mice only just a little smaller.

Mice No. 15.

Food Materials	gms.	Weights days	male	female
Ether & Alc. ext. Casein	18	1	17.	15.7
Ether & Alc. ext. Milk Powder	22	10	17.9	17.5
Corn Starch	23.8	17	17.9	16.25
Lactose	9.2	24	15.9	14.9
Röhmann's Salt Mixture	2.	31	14.	13.7
Creamery Butter	25.	38	11.2	11.
		42	10.2	9.9

This experiment shown above was with butter fat. A constant decline is well shown after 17th day. Animals died of diarrhea-like trouble. This result is contrary to Osborne and Mendel[25]. From this experiment the conclusion that the butter-fat does not contain the indispensable substance is indicated.





Mouse No. 16.

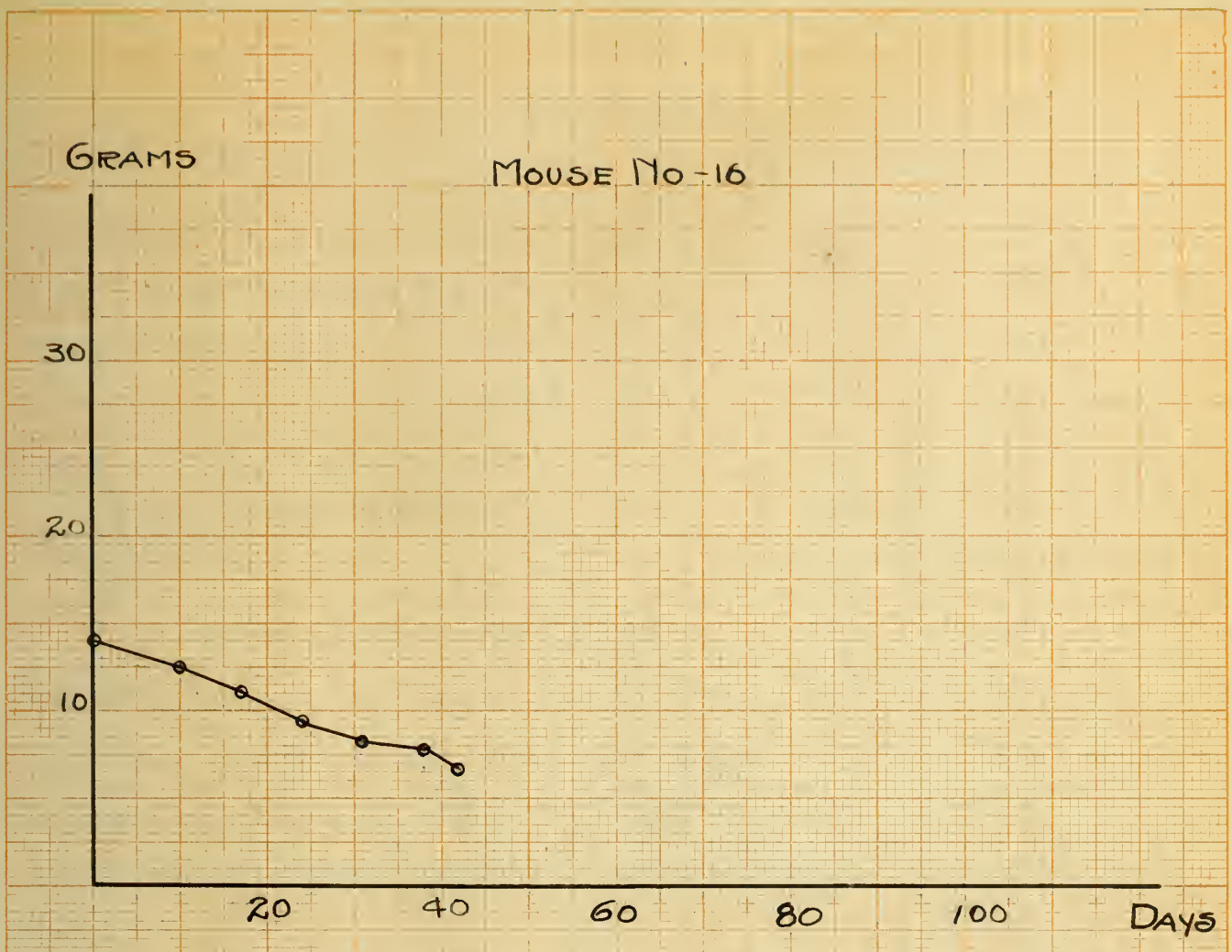
Food.		Weights.	
Materials	gms.	days	male
Ether & Alc. ext. Casein	18	1	14.3
Ether & Alc. ext. Milk Powder	22	10	12.5
Corn Starch	23.8	17	11.3
Lactose	9.2	24	9.5
Salt Mixture Röhmann's	1.	31	8.
Olive Oil	25	38	7.8
		42	6.7 dead.

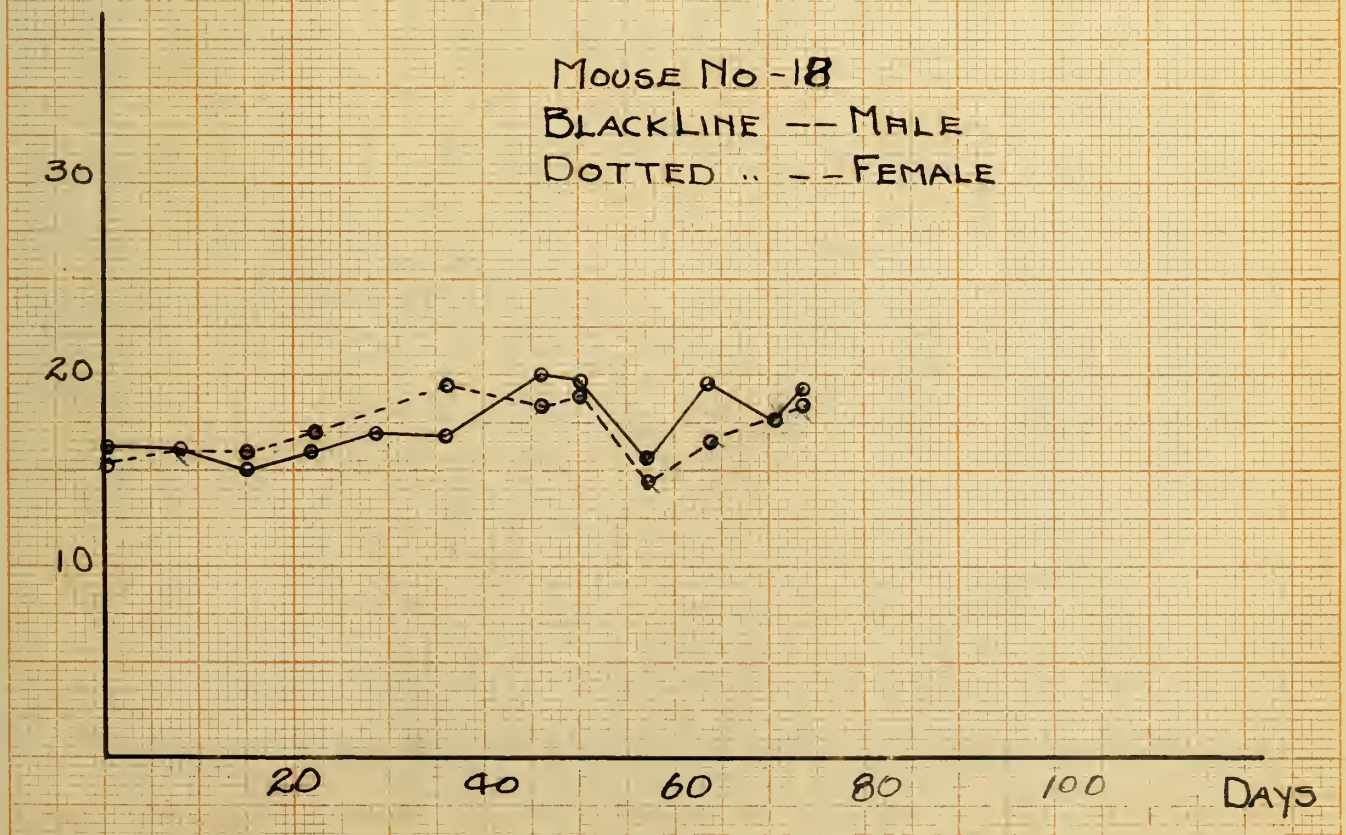
This mouse with olive oil fared no better than did No. 15 in fact not as well considering the fact that it began to decline during the first ten days while No. 15 did not.

Mice No. 18.

Food.		weights.		
Material	gms.	days	male	female.
Ether & Alc. ext. Casein	18	1	16.8	15.3
		8	16.8	16.4
Protein free Milk(pp 5.)		15	15.	16.
not extracted	29.5	22	16.	17.
		29	17.3	19.5
Starch	23.5	36	17.2	18.1
		46	20.	19.2
Lard	25.	50	19.1	19.3
		64	19.5	16.6
		71	18.	18.
		78	19.	18.

These mice were getting no lipoid material except what they received in the protein free milk. It seems to be enough although they do not look as well nor weigh as much as does No. 19. These mice were lazy and sleepy looking as compared to No. 19 and they kept their eyes closed except when disturbed.





Mouse No. 19.

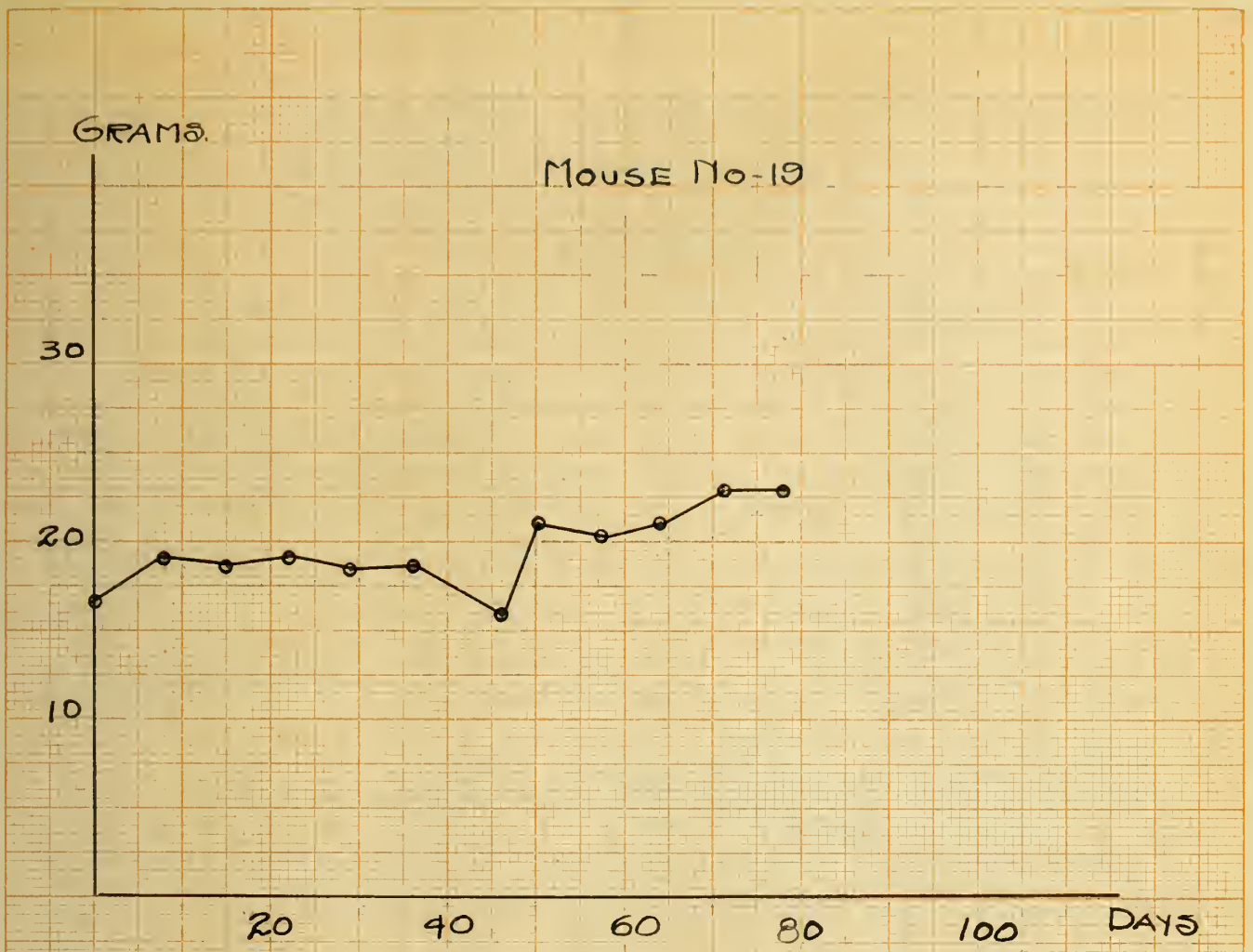
Food.		Weights.	
Material	gms.	days	gms.
Alc. & Ether ext. Casein	18	1	16.8
Protein free milk	28.2	8	18.7
Starch	23.8	15	18.2
Lard	25	22	18.7
Alc. & Ether ext. of Egg-yolk	3	29	18.
		36	18.2
		46	16.
		50	21.
		57	20.2
		64	21.
		71	22.7
		78	22.6

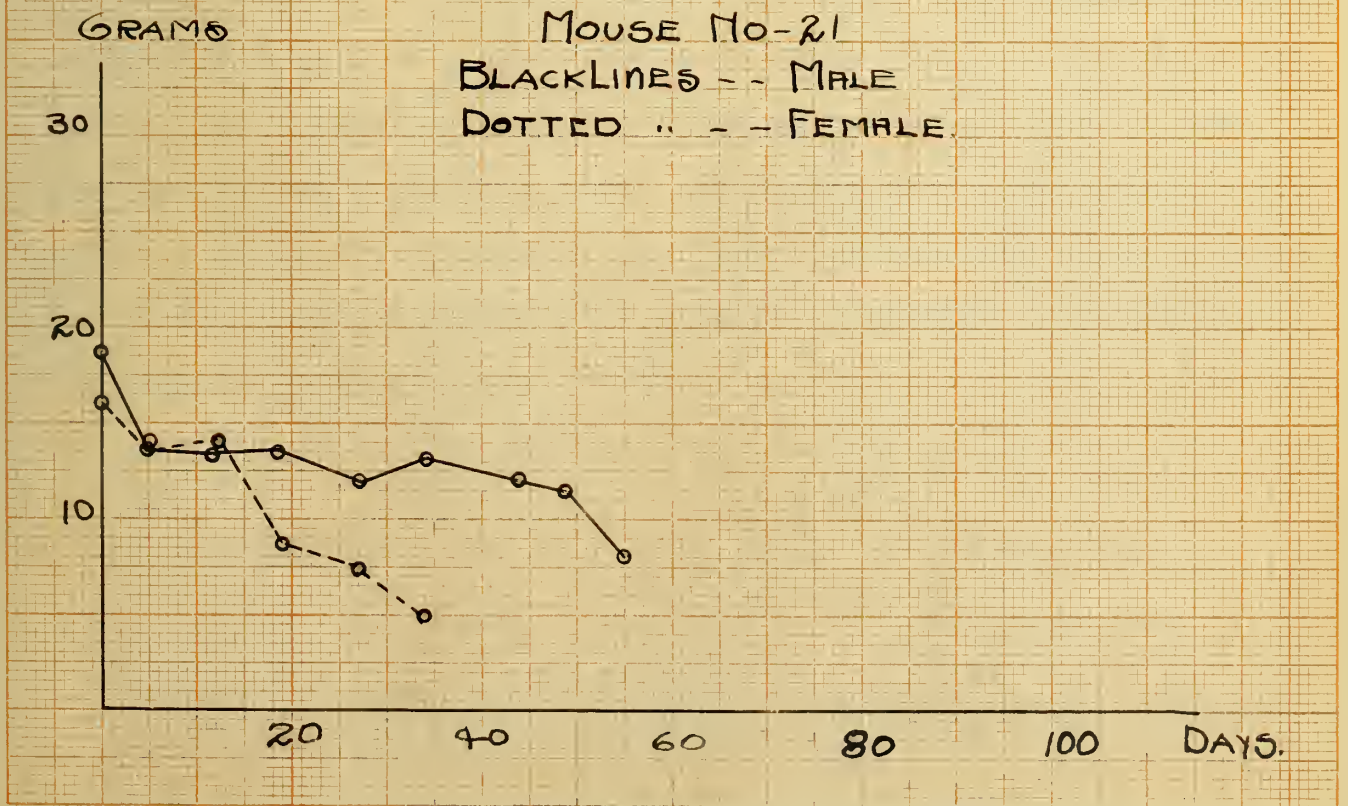
Better growth shown in this experiment than in one without the alcohol and ether extract of egg-yolk. The mouse is just as healthy as any of the mice which are receiving a variety of natural foods.

Mice No. 20.

Food		Weights.		
Material	gms.	days	male	female
Casein Alc. & Ether Ext.	18	1	17	14
		8	17	13.9
Artificial Protein free milk(pp 4)	29.5	15	17.1	13.
		22	18	12.6
		29	18.4	10.9
Starch	26.5	36	18.5	9.95
		46	17.6	9.6
Lard	26.	50	18.	8.
		57	16.5	dead
		64	15.5	
		71	12.3	
			dead	

Osborne and Mendel's artificial protein free milk failed to keep the animals alive without lipoids. It is interesting to note how much longer the male mouse maintained itself than did the



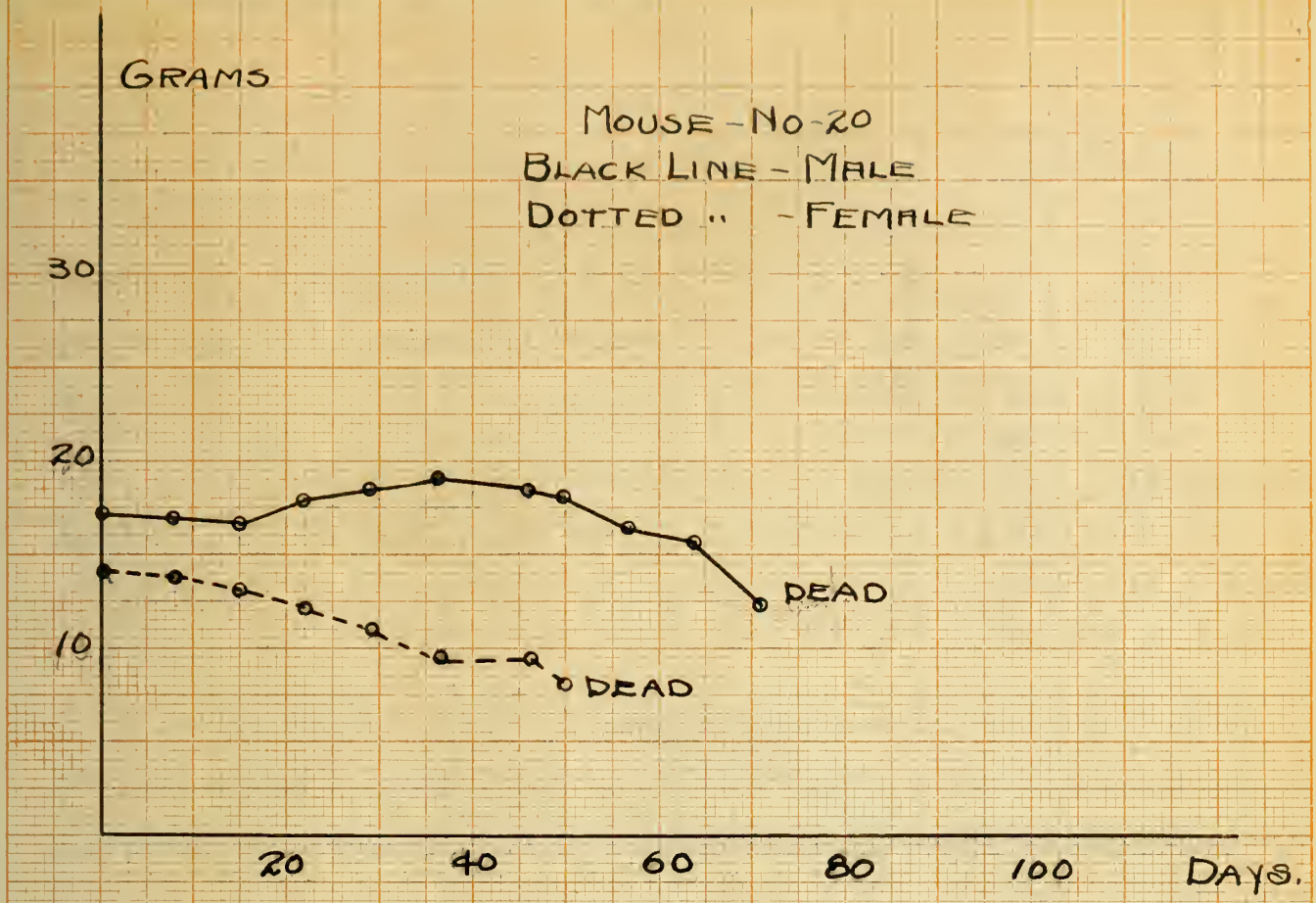


female. The male was larger to begin with and was thus better able to do without the missing substance.

Mice No. 21.

Food.		Weights.		
Material	gms.	days	male	female
Casein Alc. & Ether ext.	18	1	17.5	15.9
		5	13.2	14.
Art. Pro. free milk	29.5	12	13.2	14.
		19	13.2	8.5
Starch	26.5	27	12.3	7.5
		34	13.1	5.
Lard	26	44	12.2	dead
		48	11.4	
Alc. & Ether ext. of Egg-yolk	5	55	8. dead.	

These mice receiving alcohol and ether extract of egg-yolk showed that they were not right from the very beginning, as they were sick on the second day. These mice did not lose their weight slowly after a week or so as do mice from mal-nutrition, but they lost it within the first five days. They seemed to be lacking some kind of a substance from their food. The results obtained from this experiment are by no means certain and no conclusions can be drawn from them, especially after such experiments as Nos. 14, 19, 27, 36 and 37 have been run and show clearly that the alcohol and ether-egg-yolk extract is capable of producing not only maintainance, but growth.


GRAMS
MOUSE - NO - 20
BLACK LINE - MALE
DOTTED " - FEMALE
30
20
10
DEAD
DEAD
20
40
60
80
100
DAYS.


GRAMS.
MOUSE NO - 24.
30
20
10
20
40
60
80
100
DAYS.

Mice No. 22.

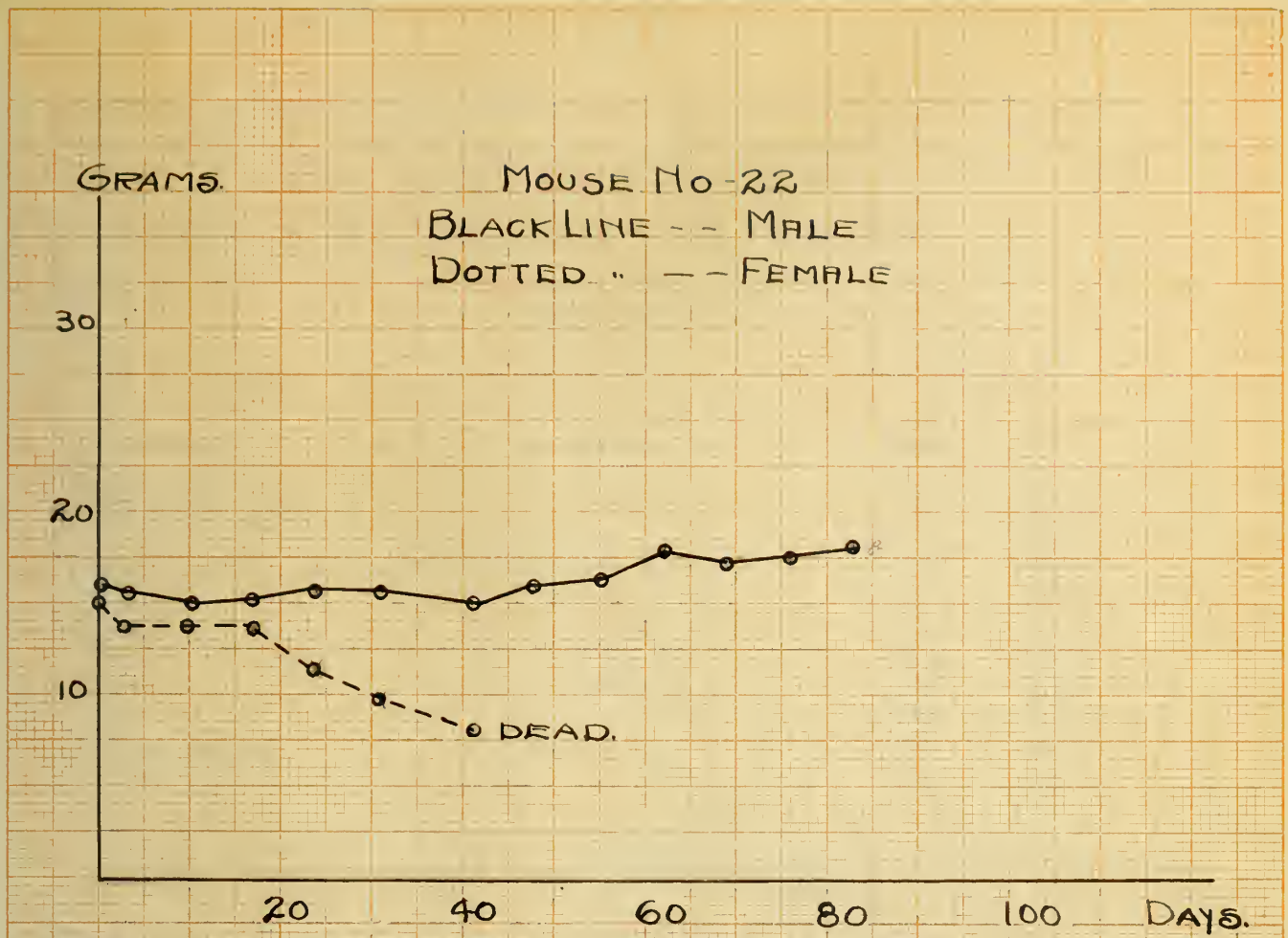
Food.		Weights.		
Material	gms.	days	male	female.
Casein Alc. & Ether ext.	18	1	16	15.2
		3	15.5	14.2
Artificial Prot. free Milk	29.5	10	15	14.1
		17	15.1	13.5
Starch	26.5	24	15.7	11.5
		31	15.8	10.
Fat	26	41	15.	8.
		55	16.5	dead
Kephalin	5	62	17.7	
		69	17.2	
		76	17.5	
		83	18.	

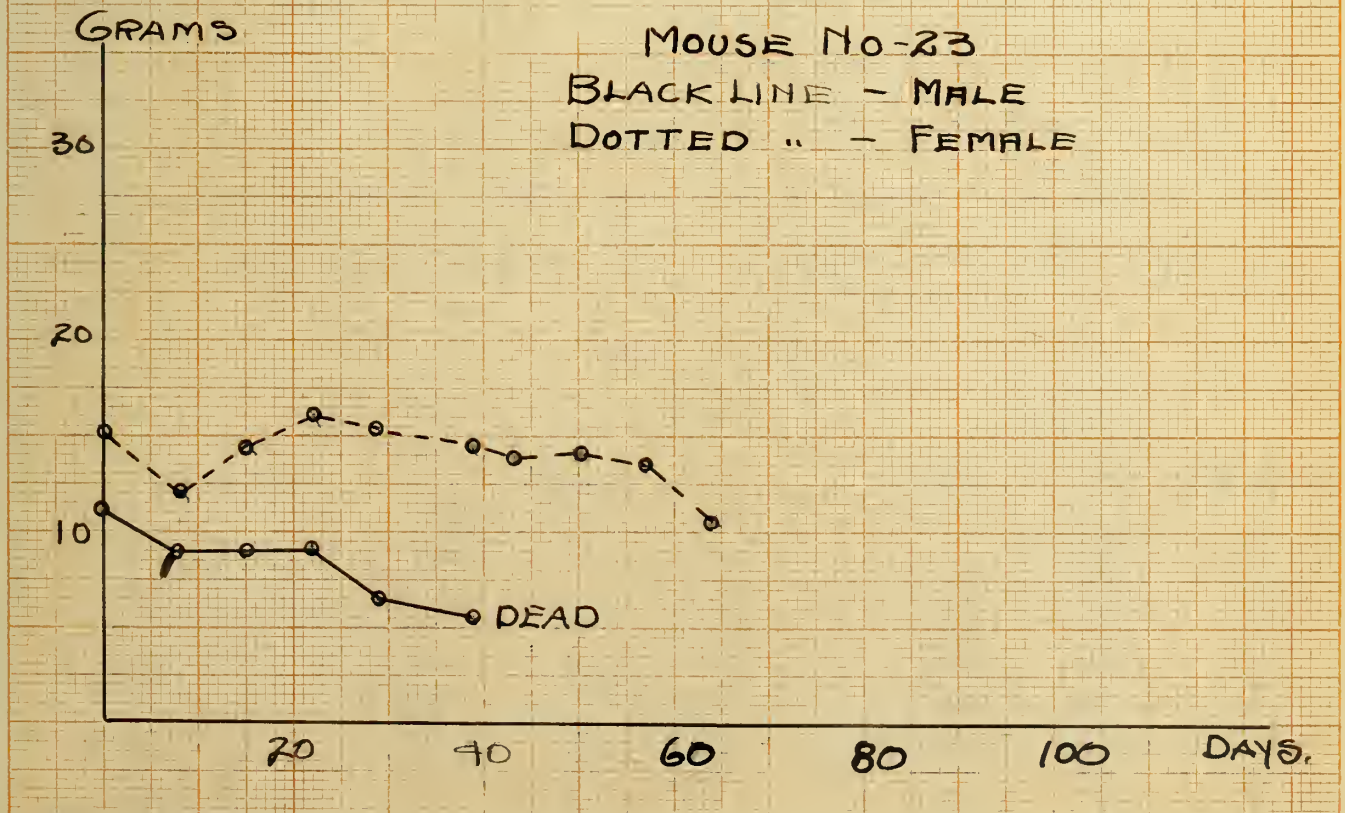
The female fell off continually in this experiment while the male shows a growth of 2 grams in 83 days which is really not growth but maintainance. This food containing Kephalin maintains the male mouse, at least, better than did the same substance in No. 7. This is easily explained by the fact that No. 22 has five grams of Kephalin whereas No. 7 only had .5 grams.

Mice No. 23.

Food.		Weights.		
Material	gms.	days	male	female.
Alc. & Ether ext. Casein	18	1	11	15.1
		8	8	12.
Art. Prot. free Milk	29.5	15	8	14.5
		22	8.1	16.4
Starch	26.5	29	6.7	15.5
		39	5.5	14.3
Lard	26	43	dead	14.
		50		14.
Lecithin	5	57		13.6
		64		10.5 dead

This food containing lecithin was not able to even maintain the animals as is especially shown in the young male mouse. This mouse receives 5 grams of the lecithin in its food and No.5 only


GRAMS.
MOUSE No-22
BLACK LINE - - MALE
DOTTED " - - FEMALE
30
20
10
DEAD.
20
40
60
80
100
DAYS.


GRAMS
MOUSE No-23
BLACK LINE - MALE
DOTTED " - FEMALE
30
20
10
DEAD
20
40
60
80
100
DAYS.

received .5 grams yet these mice (No.23) die sooner than No. 5. This shows pretty conclusively that lecithin is not the substance in question no matter in what amounts it is used.

Mouse No. 24.

Food.		Weights.	
Material	gms.	days	gms.
Alc.&Ether Casein	18	1	15.
		8	11.8
Protein free milk artificial	29.5	15	15.5
		22	16.2
Starch	26.5	29	17.1
		39	15.9
Lard	26	43	18.2
		50*	19.5
3rd Fraction	5	57	18.5
		64	16.

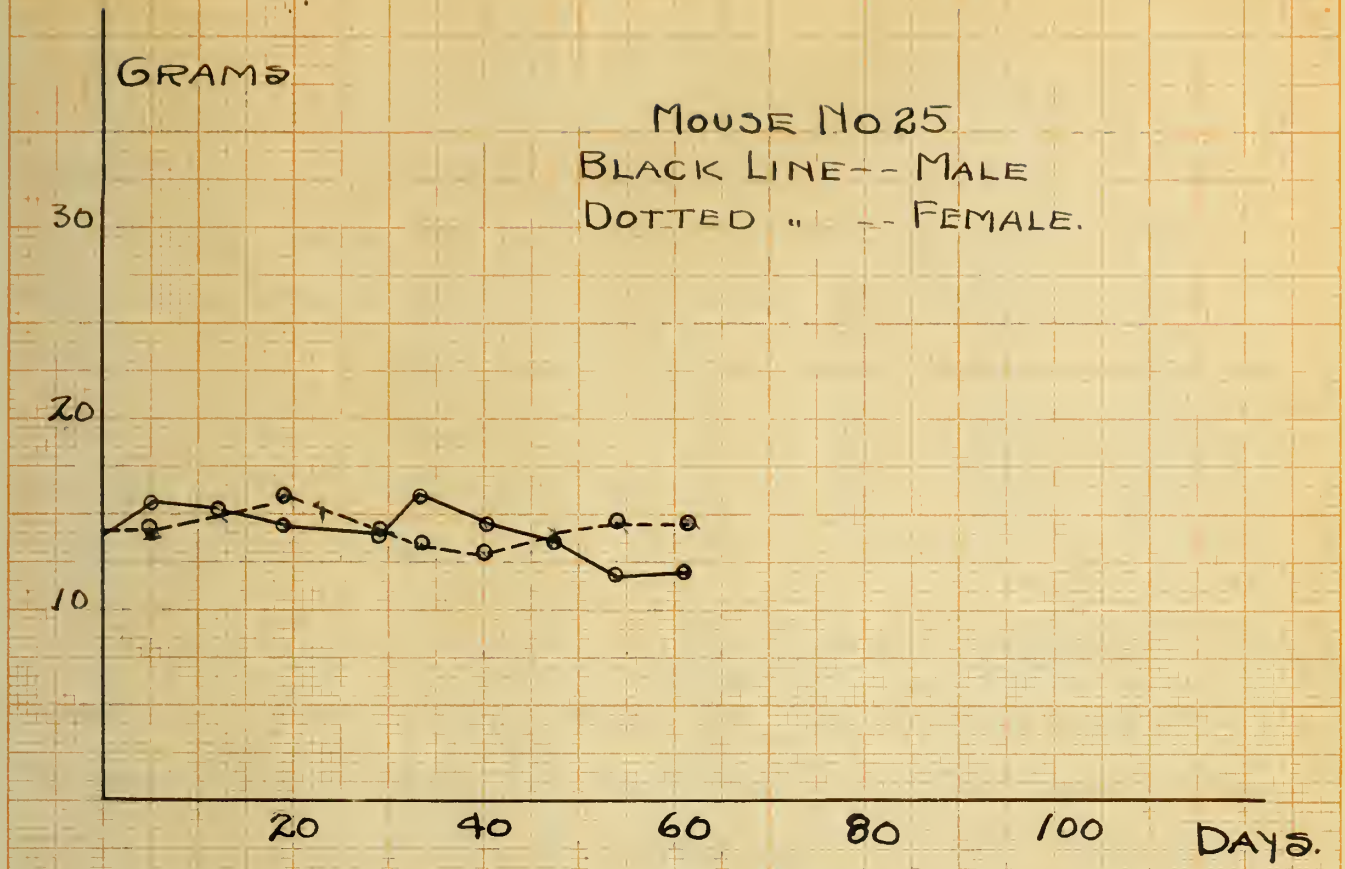
This third fraction mouse was doing pretty well until the fraction was purified on the *50th day, the animal began to decline.

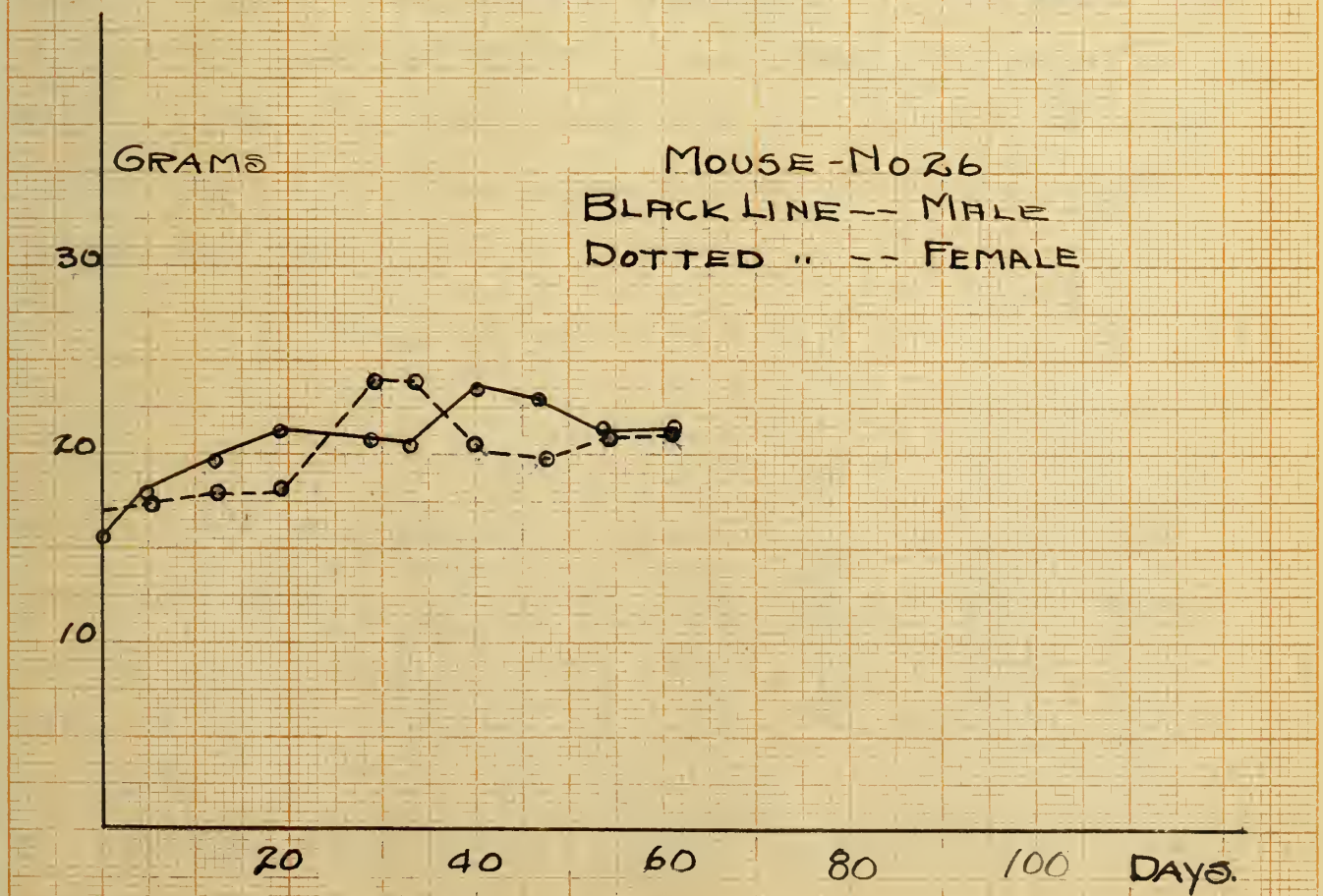
Here again the amount of third fraction was increased and the surprising thing was that the animal grew 4.5 grams in 50 days. On the fiftieth day the third fraction was purified by dissolving in acetone and evaporating the filtrate. After this was done the mouse began to loose weight and it also began to get lazy. It's hair is very rough and as a whole the mouse is in poor condition.

Mice No. 25.

Food.		Weights.		
Material	gms.	days	male	female.
Alc. & Ether ext. Casein	18	1	14.2	14
		5	15.4	14.5
Mc Collum's Salts(pp 3)	3.7	12	15.2	15.7
		19	14.5	15.9
Starch	28.3	29	14.1	14.3
		33	16.	13.4
Lard	25.	40	14.5	13.
		47	13.3	14.2
Lactose	20.	54	12.	14.4
		61	12.1	14.5

This lipoid free material is a maintainance food for practically no change has occured in 61 days. The only chance for lipoids to get into this food is by means of the casein. Still this is hardly plausible because the same casein has been used throughout these experiments.


GRAMS
MOUSE NO 25
BLACK LINE -- MALE
DOTTED " -- FEMALE.
30
20
10
20
40
60
80
100
DAYS.


GRAMS
MOUSE-NO 26
BLACK LINE -- MALE
DOTTED " -- FEMALE
30
20
10
20
40
60
80
100
DAYS.

Mice No. 26.

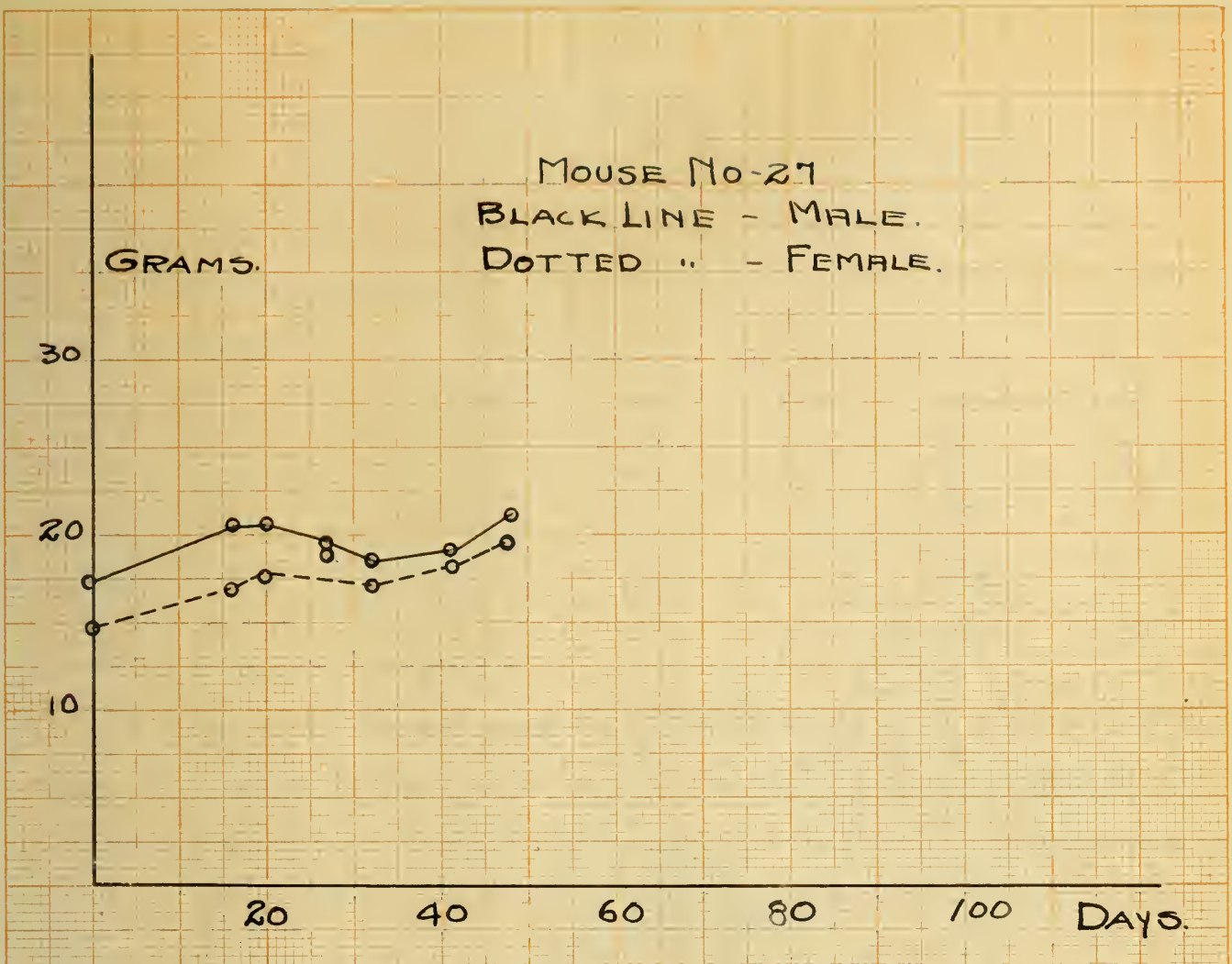
Food Materials	gms.	days	Weights male	female	
Alc. & Ether Casein	18	1	15.6	17.	
		5	17.8	17.3	
McCollum's Salts	3.7	12	19.6	17.9	
		19	20.9	18.	
Starch	28.3	29	20.4	24.	
		33	20.5	24.1	2young
Lard	25.	40	23.7	20.5	
		47	23.2	19.8	
Lactose	20.	54	21.4	21.2	
		61	21	20.9	
Dried Egg-yolk	10.				

These mice act about as well as mice not on experimental food except that they eat their young. But this is nothing important, in considering the food, for the breeding mice did the same thing when no meat was present. Fresh meat had to be given the breeding mice in order to stop them from this barbarous performance. The value of the experiment with ten grams of egg-yolk instead of one gram as in No. 6 is that it shows that the failure of No. 6 was probably due to a lack in quantity of the necessary substance already mentioned.

Mice No. 27.

Food Materials	gms	days	Weights male	female
Alc. & Ether ext. Casein	18	1	17.4	15
		16	20.6	17
McCollum's Salts	3.7	20	20.5	17.4
		27	19.5	18.8
Starch	28.3	34	18.5	16.9
		41	19.2	18.1
Lard	25	48	20.8	19.4
Lactose	20			
Hot Alc. ext. of Egg	3			

These mice did just as well as No. 26 and had only hot alcohol extract of egg-yolk. These mice are also doing well as is evidenced by their growth and production of young. Here is a very important experiment in that it shows that the alcoholic extract of egg-yolk contains the substance which cause the animal to grow.


MOUSE No-27
BLACK LINE - MALE.
DOTTED " - FEMALE.
GRAMS.
30
20
10
20
40
60
80
100
DAYS.


GRAMS
MOUSE No-28
BLACK LINES - MALE
DOTTED " - FEMALE.
30
20
10
20
40
60
80
100

Mice No. 28

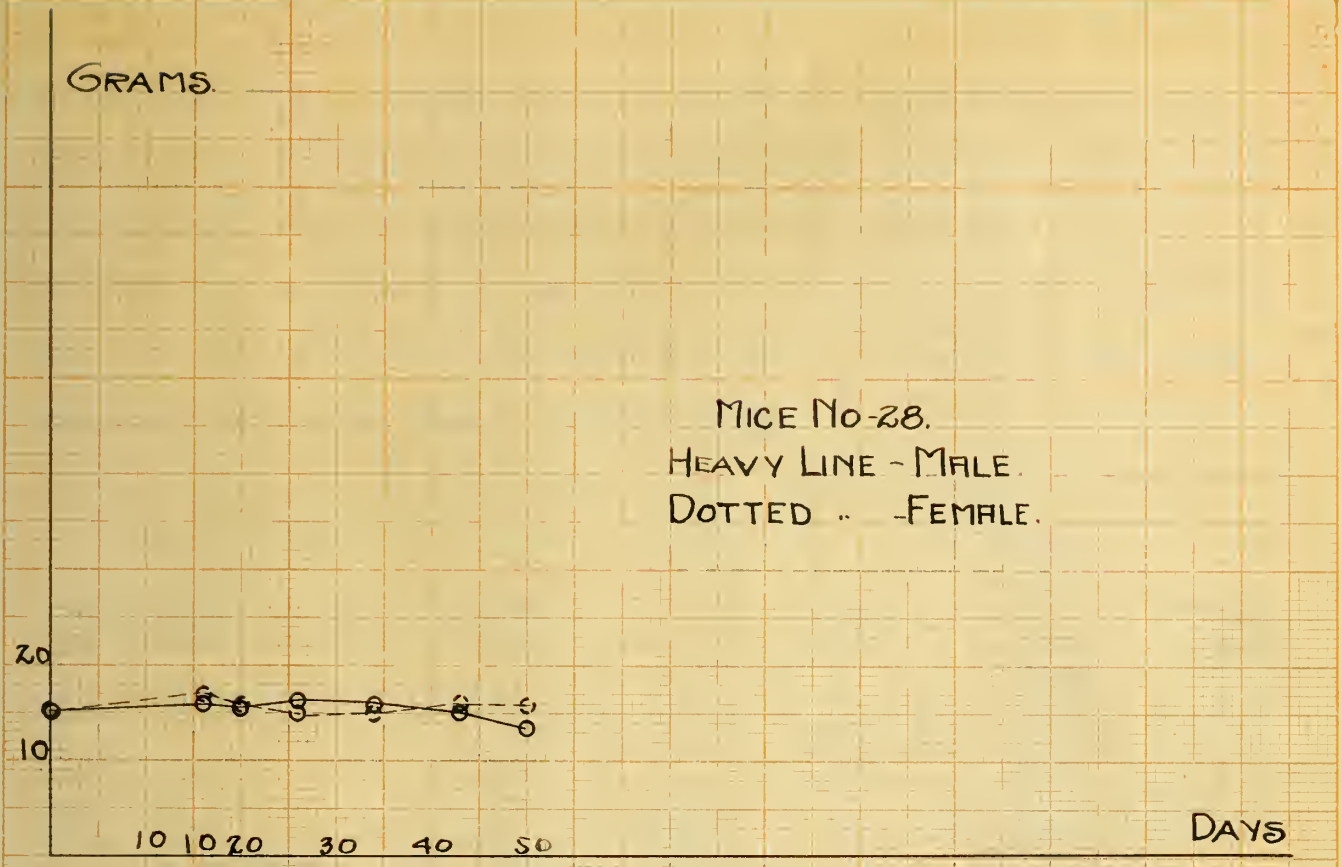
Food Materials	gms	Weights days	male	female
Alc. & Ether ext. Casein	18	1	15	15.6
McCollum's Salts	3.7	16	16	17
Starch	28.3	20	15.3	16
Lard	25	27	16.7	14.9
Lactose	20	34	16	15
Kephalin	3	43	15.5	16
		50	13.4	16

There is no growth here on Kephalin containing diet although there is a maintainance. The experiment has not run as long as the previously mentioned Kephalin experiments but from all indications, the mice will be dead in a short time for they are not faring any better than did those others in No. 22 and No. 27.

Mice No. 29

Food Materials	gms	Weights days	male	female
Alc. & Ether ext. Casein	18	1	10	11
Protein free milk ext.	29.5	15	13.2	14.8
Starch	23.5	19	13.4	15
Lard	25	26	15.2	17.2
No phosphatids		33	13.5	16.9
		40	13	15.1
		47	12	14.2

These mice have not had any phosphatid material and are sickly looking at the end of forty-seven days, although they did show a


GRAMS.
MICE No-28.
HEAVY LINE - MALE.
DOTTED " -FEMALE.
20
10
10 10 20 30 40 50
DAYS


GRAMS
MICE No-29.
HEAVY LINE -MALE
DOTTED " FEMALE.
20
10
10 20 30 40 50 60
DAYS.

surprising growth for about three weeks at the beginning of the experiment. No doubt these animals will die shortly. This experiment shows that the extracted protein free milk does not contain the elements necessary for growth. It probably does not even contain the maintainance constituents as the mice in the last three weighings have lost weight consistently.

Mice No. 36

Food Materials	gms	Weights days	male	female
Alc. & Ether Casein	18	1	14.3	9.4
		8	10.5	14.
Ext. Protein free milk	29.5	12	14.9	14.5
		19	18.5	15.4
Starch	23.5	26	19.7	looking
		33	20.5	fine.
Lard	25	40	21.5	
Egg Lipoid ppt. in cold alcohol	5			

These mice are in excellent condition. This food surely contains the substance necessary for growth. That this food contains the material which permits growth is strikingly shown by the fact that a mouse so small as the female in this experiment was able to grow so well.

Mice No. 37

Food Materials	gms	Weights days	male	female
Alc. & Ether ext. Casein	18	1	15	17
Ext. Prot. free milk	29.5	8	16	17.2
Starch	23.5	12	15.9	17.6
Lard	25	19	18	21
Soluable in the cold alcohol	5	26	18	24young
		33	17.4	18
		40	18.2	20.9

Whether No. 36 and No. 37 both owe their success to the same

substance cannot be stated but the indications are that the substance is partially soluble in the cold alcohol (pp 16) only and in this way the two parts of the alcoholic extract both contain the same substance.

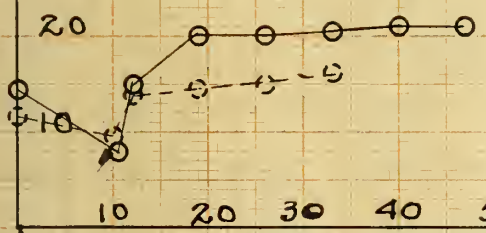
Mice No. 38

Food Materials	gms	Weights days	male	female
Alc. & Ether ext. Casein	18	1	13.2	12.9
Protein free milk heated with alcohol one day	29.5	12	17	15
Starch	23.6	17	16	16.1
Lard	25	24	17.6	17.7

This feed was made up of protein free milk heated with alcohol for one day. This alcohol was not filtered off but dried in the presence of the milk, hence there was no extraction. The mice grew as well as could be expected on artificial foods. They grew almost as rapidly as did the control mouse No. 40 and a little faster than did No. 18 which is just like this food only it is not heated.

GRAMS

MICE No-36.
FULL LINE - MALE
DOTTED " - FEMALE.



10 20 30 40 50

GRAMS

MICE No-38.
FULL LINE - MALE
DOTTED " - FEMALE.

10 20 30 40 50

Mouse No. 39

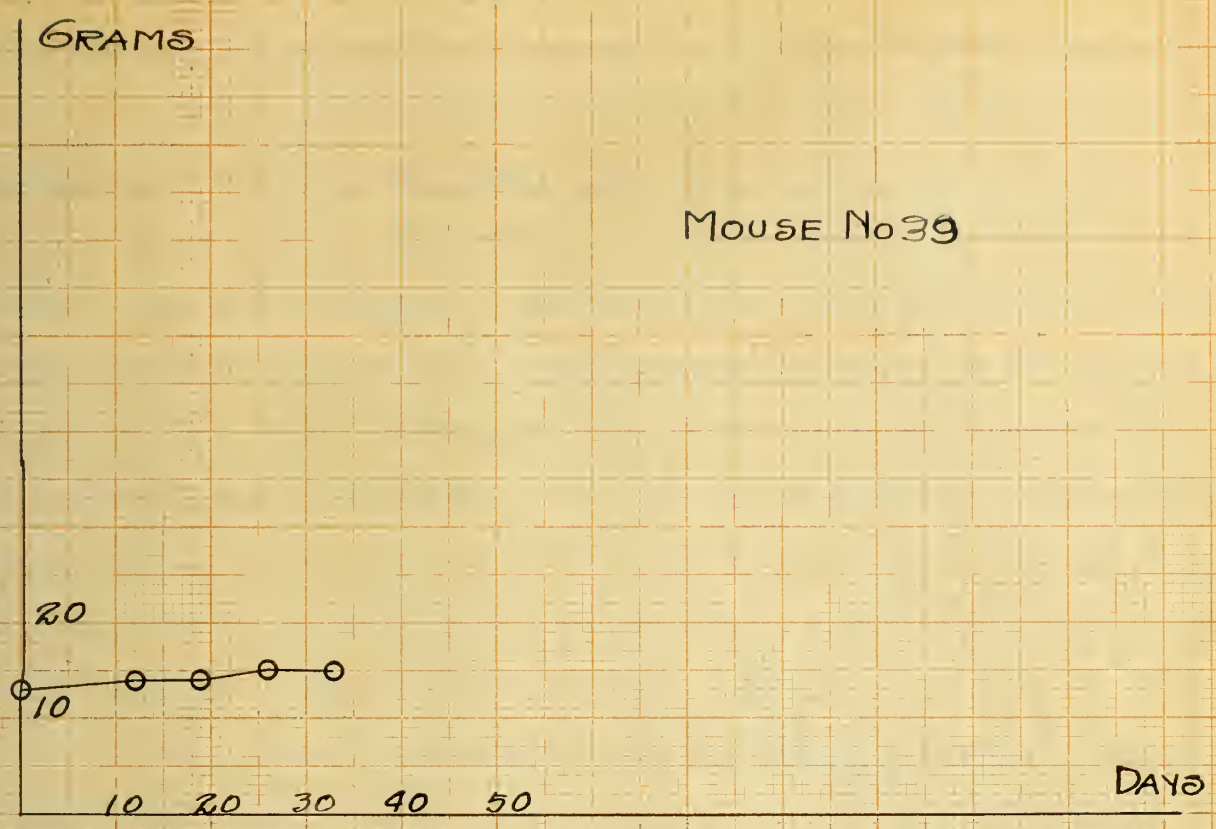
Food. Materials		Weights. days	gms.
Alc. & Ether ext. Casein	18	1	13.2
Ext. Prot. free milk	29.5	12	14.
Starch	26.5	19	14.3
Lard	26	26	15
3rd fraction	5		

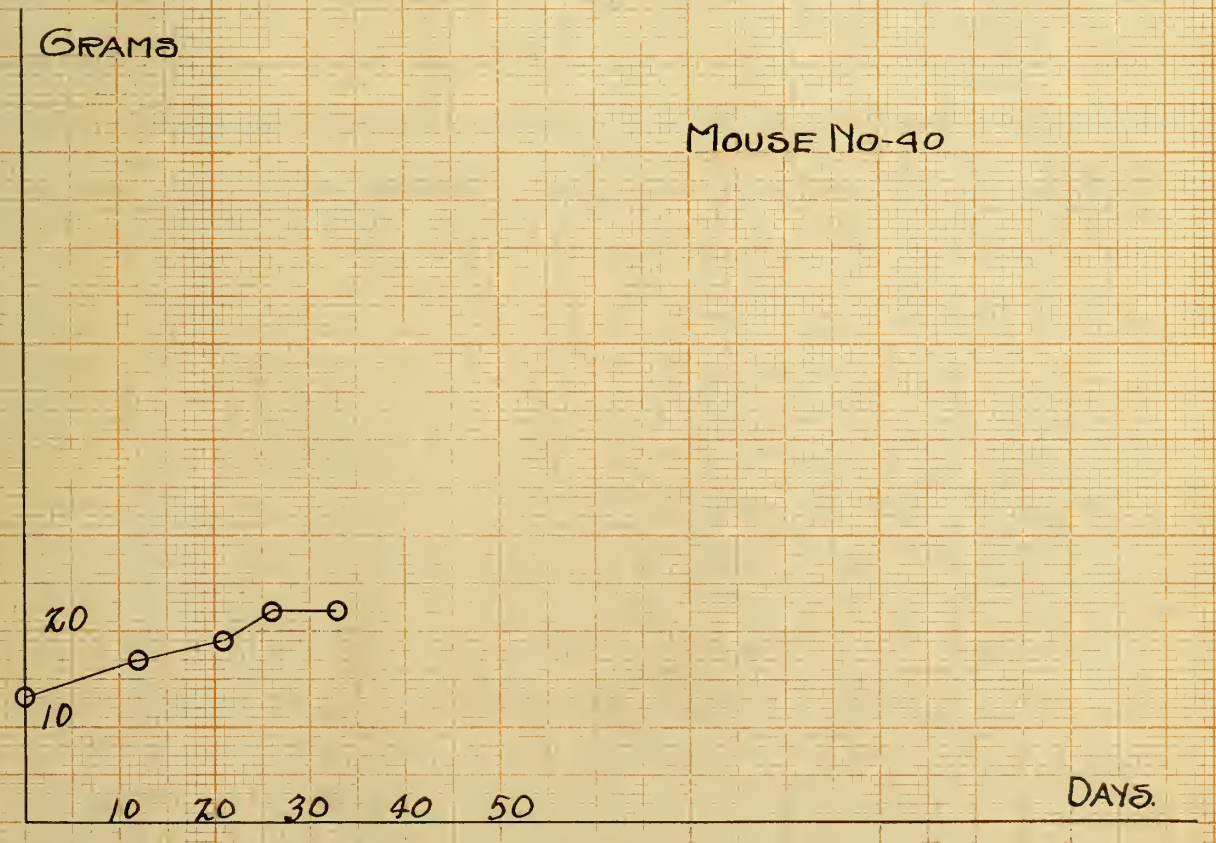
This mouse did not grow as did the mice receiving alcohol extracts of egg-yolk and the weight shown is practically nothing more than maintainance. The mouse has a rough coat and is lazy. The diet is thus shown not to be effecient in producing growth. No. 8 and No. 24 were fed this same fraction, with different salts, and no growth was obtained after a time and finally death followed. This experiment has not run long enough to determine if death will ensue, but from the looks of the mice, it is very probable that they will also die.

Mouse No. 40

Food. Materials	gms.	Weights. days	gms.
Crushed corn	x	1	13.5
Sun flower seed	x	12	17.
Bologne	x	19	21
Carrots	x	26	22

Mouse No. 40 on a natural food diet has surpassed the other mice on artificial foods, so far as speed of growth and size attained is concerned. This experiment shows that the artificial foods


GRAMS
MOUSE No 39
20
10
10
20
30
40
50
DAYS


GRAMS
MOUSE No-40
20
10
10
20
30
40
50
DAYS.

used are not quite perfect although some approach perfection.

A series of experiments heve just been started in an attempt to find out which part, if it is a part, of the alcohol extract is necessary for growth. In this series, two mice are also being fed alcohol extract of brain tissue. The extraction of the brains was done in the cold. The different fractions fed in the series are (1) cold alcohol(pp 16)extract of egg-yolk. (2) Ether(pp 16,)soluable part of alcohol. (1) extract of egg-yolk(pp 16.)(3) Warm alcohol extract(pp 16)of same egg-yolk extracted in (1). (4) Cold alcohol extract(pp 16)of brain tissue. Kahlbaun's best casein was also used in this experiment.

This series has only been going for two weeks and of course a great deal cannot be found out yet. However, it has clearly been shown that for the first two weeks all three of the different kinds of alcoholic extracts of egg-yolk permit growth, while the cold alcoholic extract of brain tissue does not do this. However, no stress can be laid upon this point since the brain tissue had previously been heated and extracted in connection with other work.

IV GENERAL DISCUSSION.

Mice 5, 6, 7, 8, and 10 are fed with untreated milk powder up until the 45th day at which time an extraction is made with alcohol and ether. All of the mice begin to loose weight immediately except No. 10. No. 6 with the whole egg-yolk begins to decline also but this is easily accounted for when it is noticed that only 1 gram of egg-yolk was fed. The amount of the necessary substance found in 1 gram of egg-yolk in 100 grams of food is doubtless so small that the mouse perished because of lack of this substance. Now No. 10 on the other hand did not start to lose weight for 17 days after the extraction so that it appears that this food had more of the necessary product in it than did any of the others in this series. No. 6 is a mixture of different substances, the lipoid fraction being one of the smallest present. When it is noticed that the alcohol and ether extracts of this lipoid fraction are still to be taken out, it is easy to see that the lipoid material in No. 6 is present in a smaller percentage than are the lecithin, Kephalin, and 3rd fraction extracts used in the other experiments of this series. These three last named fractions are shown not to contain the necessary substance or at least it is not present in sufficient amount. No. 11 is a control mouse which gets no extractives after the milk powder is extracted on the tenth day. This mouse did not grow nearly so well as the others did which show that some other substance is needed to complete the food for growth. This first series shows that the best growth is obtained with alcohol and ether extract of yolk.

No. 12 is given a food without milk powder in which the salts had to be obtained by means of Röhmann's salt mixture. This

animal clearly shows that its food is not sufficient.

Nos. 13, 14, 15, and 16 were run with the primary idea of finding out something about Osborne and Mendel's work[25] with butter-fat. No. 13 had neither phosphatid not fat in it and the mice lost rapidly. No. 14 contained lard with no phosphatid and the mouse merely maintained its weight. No. 15 with butter-fat did better than any of the others but even these died at the end of 42 days. No. 16 having olive oil for its fat barely existed for 42 days although it could hardly walk after the 17th day. According to the results obtained in this series the butter-fat seems to be but very little better than lard. However, the important thing in this series is the fact that No. 14 after being stunted began to grow and continued to grow with the alcohol and ether extract of egg-yolk when fed in a 3 gram amount to every 100 grams of food instead of .5 as in No. 10. This experiment shows conclusively that quantity of extract in No. 10 was at fault.

Mice No. 18 were fed unextracted protein-free milk and No. 19 were given exactly the same food with the addition of alcohol and ether extract of egg-yolk. No. 19 grew to be several grams heavier than did No. 18 because of the addition of this alcohol and ether extract of the yolks of eggs.

No. 38 with the heated but unextracted protein free milk showed a growth which compares favorably with No. 18 and No. 19. This fact indicates that the heating of the food used does not make it useless for growth or toxic in anyway. Of course the protein free milk was only heated 1 day whereas Stepp[20] heated 2 days in some cases. His animals were able to live upon the two days heated food, but they did not do as well as those getting untreated food.

Mice Nos. 20, 21, 22, 23, and 24 all lost weight and died in most cases or became very weak at least. The lecithin No. 23 kephalin No. 22 and third fraction No. 24 are at the best only maintainance substances when added to the lipoid free mixture. Lecithin was notably deficient. No definite conclusions can be drawn from 21 because the animals did not look right after the first day and further because there are so many other experiments in which just the opposite results were obtained.

No. 25 has no phosphatid material in it, yet the food maintains the animals perfectly although in a stunted condition. This looks very favorable for the salt mixture. No. 26, with the whole egg yolk, did not do much better than No. 27 if any and this seems to be pretty good evidence that the alcohol extract contains the necessary lipoid. Kephalin with this same mixture did not permit growth. It will be noticed that kiphalin was the best maintainer of the ether extract of egg-yolk.

Mice fed upon No. 29 are doing no growing and evidently need some growth-producing substance. This substance seems to be furnished both in No. 36 and No. 37 by the alcohol extracted fractions. No. 36 has the part of the hot alcohol extract that is precipitated in the cold and No. 37 is the part that is still soluable. Both seem to contain the substance looked for. Whether this is due to a poor separation of the two cannot be stated, but the process(pp 16)was repeated twice in an effort to purify the two fractions. The alcoholic extracts were replaced in No. 39 by the third fraction. This results in the animals maintaining its weight but not growing as do the animals having alcohol extract in their foods.

Then in order to see how a normal mouse grows under the conditions of this experiment No. 40 was run with natural food.

The fact that No. 14 grew and the other mice died which were fed parts of the ether extract namely, lecithin, kephalin and the third fraction, seems to be positive evidence that the alcoholic part of the extract in No. 14 was the cause of the growth after stunting.

No mice fed on artificial foods grew as rapidly as No. 40 does on natural food which indicates strongly that our synthetic food is not yet perfected although in some cases the growth is very nearly as rapid. It is interesting to note that where males and females were getting the same foods the males usually grow much faster.

V RESUME.

1. The quantity of phosphatid must be larger than .5 gram in 100 grams of food. 3% was found to be sufficient.

2. Milk Powder, which was not extracted, permitted growth.

3. Röhmann's Salts are not successful.

4. Butter-fat does not contain substances, at least in large enough quantities, to cause growth.

5. The alcohol and ether extract of egg-yolk started growth in a stunted mouse.

6. Protein free milk (unextracted) caused growth up to a certain weight, but this food was not as good as that fed with the addition of alcohol and ether extract of egg-yolk.

7. Heating did not effect protein free milk. The heated protein free milk permitted growth just as well as the untreated material had done.

8. Lecithin fraction seems to be the poorest growth promoter of all the fractions.

9. Both parts of the hot alcohol extract of egg-yolk (pp 16) caused growth.

10. Lipoids, or substances with lipoid solubilities, are essential for growth.

11. The hot alcoholic extract (pp 16) of egg-yolk contains this lipoid or lipoid-like substance.

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34. Milk Powder was kindly furnished free of charge by the Merril-Soules Company of Syracuse, N.Y.

35. McCollum's Salts. #187 these have not been published but given to me through the kindness of Miss Wheeler of the Department of Household Science of this University.